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A NEW FUNGUS CONCERNED IN WITHER TIP OF VARIETIES OF CITRUS MEDICA

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(WITH PLATES XXI AND XXII AND ONE TEXT FIGURE)

Following the work of P. H. Rolfs (15) the diseases of various citrus species and varieties known as wither tip or anthracnose, together with various fruit spottings, have been attributed to a well known and widely distributed fungus, *Colletotrichum gloeosporioides* Penzig.

In late years with the increased interest displayed in the citrus industry in California, a more critical and exact study has been made of these diseases which affect the various citrus species. A trouble resembling that known as wither tip in Florida was found to occur quite generally in some years and apparently to do considerable damage. The common *C. gloeosporioides* was found in abundance in connection with the disease, and the conclusion was immediately drawn upon superficial examination that the trouble was due to this fungus. But the evidence was regarded as insufficient, and in order to obtain an accurate working basis the disease was investigated at the Southern California Pathological Laboratory at Whittier, Calif. C. O. Smith failed entirely in many attempts to obtain artificial infection. C. N. Jensen was then engaged to study the problem, and he likewise was unable to make any successful inoculations with the fungus. These investigations led R. E. Smith (40) to declare that the common wither tip fungus was absolutely not a parasite on healthy citrus trees in California.

This work recalled the fact that previously McAlpine (10) had reported the fungus in question from Australia as a simple saprophyte, and that Mel T. Cook (20) from Cuba had intimated that two fungi might be concerned in the trouble. A translation of Cook's statement is as follows: "Old leaves of orange and grapefruit are frequently found with pale spots 1 cm. or more in width in which there is an abundance of spores similar to those which were found on the withered leaves of the lime; but the results up to the present time indicate that

the fungus which has been grown in pure cultures proceeding from these large spots on the leaves is not the same as that which we obtained from the withered leaves of the lime."

W. T. Horne, who had worked upon this disease with Mel T. Cook in Cuba, called the writer's attention to these earlier results, and suggested that therein lay the key to the situation. There was no question that the virulent wither tip of the lime was due to an actively parasitic fungus ; but the less virulent troubles occurring on the orange and pomelo were apparently, if we were to follow the careful work done at the Whittier laboratory, not primarily due to any fungus attack. The results of the work reported herewith have proven the correctness of the view that two fungi are concerned in the wither tip troubles. The evidence may be grouped under three main divisions, that afforded by the artificial infection experiments, the cultural differences, and the morphological differences.

INOCULATION EXPERIMENTS

The inoculation experiments, with the exception of the first three, were carried out in a heated greenhouse. All necessary precautions were taken to guard against any accidental infections which might throw doubt on the results obtained. Small seedling potted trees were used in these experiments except in the case of the tangerines and lemons which were budded trees. In the first experiment the plants were simply covered with a bell-jar and atomized with water daily for a week but this method did not keep the humidity of the atmosphere in the bell-jar up to the point of saturation as was desired. Accordingly, in subsequent experiments the pots were set in moist sand beds and the jars allowed to rest on the moist sand. In this way it was found possible to keep the atmosphere under the bell-jar practically saturated throughout the experiment, thus furnishing the best possible conditions for infection. Inoculation was either effected by atomizing with a spore suspension of the fungus or by painting on such a suspension with a camel's hair brush. In the former case the plants were atomized with water daily in order to keep the foliage moist, in the latter case they were atomized as often as was thought necessary. Painting the spore suspension on with a camel's hair brush was preferred, since it was found easier to approximate natural conditions of infection in this way and to prevent the fungus escaping control.

The results of these experiments are assembled in the accompanying tables and very little explanation is necessary in connection with

them. It will be seen that in cases where material from Florida or California was used, or when strains originating from such material were used, the results were uniformily negative. From the California standpoint this would be the expected result, if we are to judge from results reported from the Whittier Laboratory. In Florida, however, a virulent form of wither tip undoubtedly does occur on the lime; but, although such material was requested from several parties none was received. The Cuban strains which originated from lime material gave uniformly positive results on the lime, and in one instance an unmistakable infection on the lemon. The exception to these uniformily positive results was afforded by Experiment XXVI. The explanation here seems to be fairly simple. An examination of the drops of moisture on the leaves after seven days showed that very few of the spores had germinated, and these sent out very weak germ tubes. The heat had been shut off from the greenhouse at this time and cold damp weather prevailed during the time of the experiment. Unfortunately, time did not permit of accurate determination of the relation of germination to temperature, consequently this point must remain in doubt. The repetition of this experiment under more favorable conditions, reported as Experiment XXVII, gave the usual positive results.

The control plants in all cases were free from infection.

The extent of parasitism on the lemon was not fully demonstrated in the experiments, but the parasitism in the one positive case was unmistakable. The lemons used were rather large budded trees, too large in fact to use satisfactorily with the facilities at hand; and this, coupled with the difficulty of obtaining exactly the right kind of tender young growth at just the time it was needed, could very easily account for the negative results recorded in Experiment XV and on the one lemon tree in Experiment XXVII. If conditions are in every way favorable, the virulent fungus will undoubtedly be found capable of active parasitism on the lemon.

This brings to mind the interesting fact of the close relation botanically of the host plants, a relation that has been called to mind in the title of this paper. According to Webber (43) the sweet lime, the sour lime, and the lemon are to be regarded as forms or varieties of one species, *Citrus medica* L.

Wither Tip of the Lime. The lesions caused by this pathogenic fungus closely follow the descriptions given by Rolfs (15) and Mel T. Cook (20) on the lime. With the plants in moist chambers infection is first indicated by a dark water-soaked appearance of the affected part of the leaf. Under very moist conditions a very sparse

TABLE I. CUBAN STRAINS

No.	Date	Trees inoculated	Controls	Manner	Material used in inoculation	Results
IV	Oct. 19, 1911	2 sweet oranges 2 sour oranges 1 pomelo 1 sour lime	None	Brush	Spores taken from scraps of lime leaves and twigs and orange leaves from Prof. Earle, Herradura, Cuba	Negative " " Positive
V	Oct. 31, 1911	1 sour lime 1 sour orange 1 sweet orange 1 pomelo	None	Brush	Spores taken from orange leaves from Prof. Earle, Herradura, Cuba	Negative " " "
X	Nov. 18, 1911	2 sour limes	1 sour lime	Brush	Spores taken directly from an infected leaf of the lime used in Experiment IV	Positive
XI	Nov. 24, 1911	1 sour lime 1 sour orange 1 sweet orange 1 pomelo	None	Brush	A pure culture of a strain of C. gloeosporioides isolated from the material used in Experiment IV	Negative " " "
XIII	Dec. 1, 1911	2 sour limes	1 sour lime	Brush	A pure culture isolated from the leaf used in Experiment X	Positive
XV	Jan. 30, 1912	2 sour limes 2 sour oranges 2 tangerines 2 lemons	1 sour lime 1 sour orange 1 tangerine 1 lemon	Atomizer	A pure culture of the strain used in Experiment XIII	Positive Negative " "
XVI	Feb. 3, 1912	2 pomelos 2 sweet oranges	1 pomelo 1 sweet orange	Atomizer	A parallel pure culture to that used in Experiment XV	Negative "
XVII	Feb. 7, 1912	2 sour limes 2 sour oranges 2 sweet oranges 2 pomelos	1 sour lime 1 sour orange 1 sweet orange 1 pomelo	Brush	A parallel pure culture to that used in Experiment XV	Positive Negative " "
XXI	Feb. 27, 1912	2 tangerines	1 tangerine	Brush	A parallel pure culture to that used in Experiment XV	Negative

XXIV	Apr. 2, 1912	2 sour limes	1 sour lime	Brush	A pure culture from one of the limes in Experiment XVII	Positive
XXV	Apr. 11, 1912	2 sour limes	1 sour lime	Brush	Spores from lime scraps from Prof. Earle, La Gloria, Cuba	Negative
XXVI	Apr. 11, 1912	2 sour limes	1 sour lime	Brush	A pure culture isolated from material from Mr. Van Hermann, Santiago de las Vegas	Negative
XXVII	Apr. 20, 1912	2 sour limes 2 lemons	1 sour lime 1 lemon	Brush	A pure culture from the strain used in Experiment XXVI	Positive * "

* In Experimeut XXVII the positive result consisted in an infection of three young leaves of one of the lemons. The other lemon, as well as the control, was not affected in any way.

TABLE 2. CALIFORNIA STRAINS

No.	Date	Trees inoculated	Controls	Manner	Material used in inoculation	Results
I	Sept. 12, 1911	1 sour lime 1 sweet orange 1 sour orange 1 pomelo	None	Atomizer	Spores taken directly from tangerine leaves from Duarte, Cal.	Negative " " "
II	Sept. 21, 1911	1 sour lime 1 sweet orange 1 sour orange 1 pomelo	None	Brush	A pure culture isolated from spotted lemon leaves from Whittier, Cal.	" " " "
III	Oct. 10, 1911	1 sour lime 2 sweet oranges 2 sour oranges 1 pomelo	None	Brush	A pure culture isolated from spotted lemon leaves from Covina, Cal.	" " " "
XVIII	Feb. 2, 1912	2 sour limes 2 sweet oranges 2 sour oranges 2 pomelos 2 lemons	1 sour lime 1 sweet orange 1 sour orange 1 pomelo 1 lemon	Brush	A pure culture isolated from the material used in Experiment I	" " " " "

TABLE 3. FLORIDA STRAINS

No.	Date	Trees inoculated	Controls	Manner	Material used in inoculation	Results
VI	Oct. 31, 1911	1 sour lime 1 sweet orange 1 sour orange 1 pomelo	None	Brush	Spores taken direct from a pomelo twig and piece of pomelo rind from H. S. Fawcett, Gainesville, Fla.	Negative " "
VII	Nov. 16, 1911	1 sour lime 1 sweet orange 1 sour orange 1 pomelo	None	Brush	Spores taken direct from a large spotted pomelo from H. S. Fawcett	" " " "
XII	Dec. 1, 1911	2 sour limes	1 sour lime	Brush	Spores from a spotted lemon from H. S. Fawcett, Gainesville, Fla.	"
XIV	Jan. 24, 1912	2 sour limes	1 sour lime	Brush	Same as in Experiment XII	"
XIX	Feb. 19, 1912	2 sour limes 2 sour oranges	1 sour lime 1 sour orange	Brush	A pure culture from the material used in Experiment VI	" "
XX	Feb. 27, 1912	2 sour limes 2 sweet oranges 2 sour oranges 2 pomelos	1 sour lime 1 sweet orange 1 sour orange 1 pomelo	Brush	A parallel pure culture to that used in Experiment XIX	" " " "
XXII	Mar. 6, 1912	2 sweet oranges 4 sour oranges 2 pomelos 2 tangerines	1 sweet orange 2 sour oranges 1 pomelo 1 tangerine	Brush	A parallel pure culture to that used in Experiment XIX	" " " "
XXIII	Mar. 14, 1912	4 sour limes 2 sweet oranges 2 pomelos 2 tangerines	2 sour limes 1 sweet orange 1 pomelo 1 tangerine	Brush	A parallel pure culture to that used in Experiment XIX	" " " "

white mycelium may appear on the surface of the affected portion. The spot then enlarges rapidly, the leaf retaining the water-soaked appearance until it is practically entirely destroyed, and then withering away. This is the case when the atmosphere is practically saturated.

Under ordinary moisture conditions the spot enlarges very slowly. The water-soaked appearance gives way to a bay color, which fades thru Saccardo's dark and light brick-color until in mature spots only a very faint honey-color remains. A dark brick-colored border often, but not always, persists. The acervuli appear at about the time the the water-soaked appearance begins to fade. They are at first very distinct bright flesh-colored points, which deepen in color as the spot becomes older, until finally they take on a rusty-brown color. They never become black, and they are most abundant on the upper surface of the spot.

Infection in the inoculation experiments usually took place along the margin of the leaf, which further accords with the report of Rolfs concerning wither tip of the lime. Under especially favorable conditions, however, infection may occur at any place on the leaf. Such internal infections, under ordinary conditions of humidity of the atmosphere, commonly develop very slowly, and the dried ashen portion often cracks, thus giving the leaf the appearance of having been torn by thorns. This effect is enhanced by the curled and distorted appearance of affected leaves. In our work infections developed only on the very young tender leaves, the older leaves apparently not being susceptible to attack.

Infection sometimes occurs on a tender stem. In such cases the infection may take place at a particular point on the stem, say two or three inches from the terminal bud, and completely cut off the shoot at that point causing the part beyond to fall over limply, wither, and die. Rolfs has described an anthracnose of the lime which in every way agrees with the stem infection that has occasionally developed in the inoculation work.

Under favorable conditions the infections are first plainly apparent in from four to seven days after inoculation. The acervuli appear about two days later than this. Infection always follows promptly after inoculation, and practically no laggard infections develop after the first week. Experiments during cooler weather, however, sometimes take longer. In Experiment XIII, for example, the infections had not developed to any extent until the eleventh day after inoculation.

It was impossible with the material at hand to investigate such phases of the problem as bloom dropping, fruit canker, scab or

verrucosis, or the role which the fungus might play in the various ripe rots of citrus fruits. Wither tip infections of young lime fruits are, however, very probably caused by this fungus. The observation of Cook and Horne (23) that infection of sour lime did not take place after the fruits were well set or after the leaves and twigs had hardened somewhat is in accordance with the observations we have made in the inoculation work and, therefore, in a measure supports this opinion. It is our opinion, however, that such ripe rots as are caused by a fungus of this type are due to the common wither tip fungus. This opinion is based on the indications of an unfavorable effect of lower temperatures on the development of the virulent fungus as shown in Experiment XXVI.

CULTURE STUDIES

In the culture work the usual methods were employed. In all cases strains were established by isolating a single spore in a hanging drop and carefully watching its germination and subsequent development. The strains were grown in triplicate and the results confirmed by growing different strains of the same fungi on the same media. Only macroscopic characteristics of growth were noted. Cultures were kept at laboratory temperature, varying from 10 to 20 degrees Centigrade, usually, however, about 15 degrees. Growth was studied on dextrose-peptone agar, steamed rice, prune juice and bread, steamed citrus leaves, bean pods in tubes, and potato plugs.

On Agar. Dextrose-peptone agar made up according to the following formula was used :

Shredded agar	15 grams
Peptone	10 "
Dextrose	10 "
Sodium chloride	5 "
Water to make	1 liter.

The medium was not titrated. About 10 cc. of this medium was poured into a 10 cm petri dish and a piece of a pure culture planted in the center.

The common wither tip fungus forms at first a fluffy colony of white mycelium faintly tinged with pink. There is an indistinct radial and zonal distribution of growth. Within a week the sporodochia begin to appear. These are small and black and soon send out salmon-colored masses of spores. These sporodochia may be distributed more or less concentrically but this is usually not noticeable on an old plate. Growth is at the rate of about 2 cm. a week.

The virulent fungus grows somewhat less than half as fast and after the first week develops no new characteristics so far as have been observed. When the colony is about 1 cm. across—five to seven days after planting—the central 4 mm. is practically a confluent mass of spores, and surrounding this is a rather scanty pink-tinged mycelium. Subsequently, as the fungus grows, this mass of spores extends up to within 3 or 4 mm. of the edge. With age the edge of the colony may become somewhat crenated. No black sporodochia or other structures have been observed even in old plates. A very characteristic feature, but rather difficult of description, is the tufted appearance of the surface of the colonies.

On Steamed Rice. Ten grams of rice were steamed up with 50 cc. distilled water in a 200 cc. Erlenmeyer flask. This furnished a very clean desirable medium, especially for the development of color characteristics, and on this medium the differences between the two fungi were most marked.

The growth of the common wither tip fungus has been found to be somewhat more variable than that of the virulent fungus, depending upon the age of the strain as well as upon individual differences between strains. Typically, minute scattered sporodochia first appear in the fluffy white mycelium, and these soon become so abundant as to give the colony a black appearance. Soon, however, a white superficial mycelium develops which later becomes appressed and thickly matted, effectually masking the black pellicle. A culture one month old will have a fungus growth over the entire surface of the medium. There will be a black pellicle underlying the whole colony and pushing thru in irregular areas at the bottom of the flask. The colony will have a black appearance up to within 0.5 cm. of the side of the flask, where there will be a narrow zone in which the pellicle has not yet turned black, but is stained a bright flesh-color, practically the same color that is so conspicuous in the case of the virulent fungus. In this narrow zone there will be scattered black sporodochia, some of them nearly 1 mm. across. Overlying the black pellicle is a fairly heavy white or grayish entangled growth of mycelium. This is not at all appressed and is not produced uniformly over the whole surface, but in such a way as to give the colony a netted appearance. Originating from the pellicle are numerous flesh-colored spore masses about 1 cm. across which show thru the spaces in the superficial growth of mycelium. There is no staining of the medium up to this time.

At three months the surface will have become appressed and thickly matted masking the black pellicle. The irregular projections of the pellicle at the bottom of the flask will be more numerous. Around

the edge of the colony the previous description will still hold, although the color production will be much less marked. The greatest change is in the medium, which will have a dirty water-soaked appearance and will be in process of slow liquefaction.

Colonies four months old will have changed very little. The liquefaction and shrinking in volume of the medium will be more marked, and the color of the medium will approach Saccardo's drab, but somewhat lighter.

The growth of the virulent fungus is strikingly different. There is first produced an inconspicuous white mycelium followed close up to the edge of the colony by a confluent bright flesh-colored layer of spores. This growth continues until in about a month the entire surface of the medium is covered.

Cultures one month old show the characteristic confluent layer of flesh-colored spores covering the entire surface of the medium. At this time a superficial mycelium is beginning to develop, but does not as yet cover the entire surface. Thus far it is only a scanty surface growth, the spore masses beneath it appearing darker than those free from it. There are suggestions of pellicle formation around the edges. There is a bright flesh-colored line about 1 mm. broad bounded below by a narrow white line. At the bottom of the flask there is just a suggestion of color development in the medium, a faint isabel tinge.

At the end of three months the superficial mycelium has developed more abundantly and has become matted and appressed. Extensions of the pellicle in irregular areas to the bottom of the flask have developed and some of these show a bay-colored line where they meet the glass. Others, and those around the edge, are white, faintly tinged with pink. The medium at this time has deepened to a light brick color. Liquefaction and shrinking begin at this stage.

A month later the cultures will show only slight changes. The former confluent layer of spores will show thru the superficial mycelim as light brick to almost bay-colored areas. The medium will be stained nearly a bay color, and liquefaction and shrinking will have continued along the lines indicated above.

On Prune-juice and Bread. Enough stale bread was broken up into a 200 cc. flask to soak up well with 50 cc. of prune-juice, made by boiling 120 grams of dried prunes in water, and then making the mixture up to 1 liter and filtering.

Growth on this medium bears out the general characteristics already noted above. The common wither tip fungus first produces a dense colony of cottony mycelium. This is soon followed by production of a black or greenish-black substratum in the center of the colony. The white fluffy growth will extend over the entire surface of the medium

in three weeks, and at that time a 1.5 cm. zone in the center will show this black substratum. In another month the black stromatic pellicle will underlie the whole surface of the colony. Masses of salmon-colored spores are produced here and there. Ultimately the surface growth of mycelium melts down to a wet shiny black crust.

The virulent fungus grows at about the same rate as the common wither tip fungus. At first the colony is much like that produced on rice, a layer of spores overlaid with a scanty white mycelium and surrounded by a 5 mm. zone of white mycelium. This growth covers the surface of the medium in about three weeks, and then a denser development of mycelium takes place over the surface until the colony asumes a deeply convoluted, matted appearance. The color showing thru is brown instead of black; and no black is seen at the edges where the colony meets the glass side of the flask. Instead, the pellicle is deeper brown in spots at such places. Brick-colored drops of liquid are exuded and these melt the surface down to a wet brick-colored pellicle with darker dirty bay-colored areas. The bottom of the flask shows a change in the medium at this stage, altho slight. It has become somewhat lighter and brighter in color than it was originally, as tho some yellow had been splashed thru it.

On Bean Pods. Canned string beans were thoroly washed and then placed in tubes and sterilized. They furnished a very satisfactory medium for spore production, and practically all pure culture inoculations were made from bean pod cultures.

Miss Stoneman (9) has described the growth of *Colletotrichum gloeosporioides* on bean pods, and observations made in this work agree with those she has noted. Growth at first consists of a fluffy white mycelium producing numerous but minute black sporodochia. This is followed by production of a black stroma overlaid and obscured by the production of a closely matted growth of white mycelium. Masses of spores originate from the sporodochia, and are produced in such abundance that the salmon-colored confluent spore masses may be 1 cm. or more long. Ultimately, however, the white superficial growth of mycelium covers them. At this stage the black stroma shows thru wherever the colony touches the glass.

The growth of the virulent fungus does not differ from that already noted for other media. A fluffy colony of whitish mycelium is first produced, followed by the production of a confluent mass of spores at the middle. As growth proceeds this mass of spores follows up within about 7 mm. of the edge of the colony until ultimately the entire surface of the culture is covered with the flesh-colored mass of spores. After this very little change takes place. A scanty white superficial mycelium may be produced, but more often the colony merely becomes

slightly darker with age until finally it dries up. No black stroma is produced.

On Citrus Leaves. Citrus leaves, mostly pomelo, were steam sterilized with water in a 500 cc. flask. This medium brought out very few characteristics of any importance and was rather unsatisfactory in general. The common wither tip fungus produced acervuli showing the characteristic dark color and characters of *C. gloeosporioides.* The virulent fungus produced acervuli which were lighter in color than those of the common wither tip fungus and had no suggestion of the black stroma produced in that fungus. These differences are described subsequently with the morphological characters. No indications of a perfect stage were discovered. Following the development of the acervuli a superficial mycelium is produced which forms a silvery-gray appressed covering over the entire leaf surfaces. Black sporodochia are then formed beneath this mycelium and push up thru it, forming ultimately a black roughened crust over the surfaces of the leaves. At the end of one year in large Mason jar cultures this stage had not yet reached completion. No cultures of the virulent fungus old enough to show any further stages of development than the acervuli were at hand.

On Potato Plugs. Potatoes were steamed, then cut into strips, and steam sterilized in tubes. This medium proved exceedingly unsatisfactory from the standpoint of growth for both fungi. After two weeks the wither tip fungus had produced a colony 3 cm. long; the virulent fungus, one about half as long. At the end of two months growth in either case did not exceed 4 or 5 cm. As in other cultures the wither tip fungus developed a black stroma; the pathogenic organism did not. However, the distinction was not so evident on account of a dark staining of the medium in the case of both fungi. At the end of two months the colonies looked almost alike superficially, a closely compacted white growth with a slightly rosy tint.

It will be readily seen from these descriptions that there is only a general similarity in growth of the fungi, and that the differences are sufficient to distinguish them by culture characteristics alone. Steamed rice has proved an especially desirable and easily prepared medium, and the absolute difference in behavior of the two fungi on this medium is evidence enough of their distinctness. Disregarding minor differences which have been noted thruout the study, there is one distinguishing feature which stands out clearly on all media on which the fungi have been studied, namely, the virulent fungus has never been found to develop a black stroma or pellicle as the common wither tip fungus does.

MORPHOLOGICAL STUDIES

The morphological differences between the two fungi have proven no less marked than the parasitic and cultural differences. The fungus which has given uniformly negative results in our work has been found to agree with the description of *C. gloeosporioides*. This is in accordance with the judgment of Woods, Rolfs, R. E. Smith (40), Essig (37), and others regarding the identity of the fungus found in California and is in opposition to the view of Mel T. Cook (20) that virulent wither tip of the lime was to be attributed to *C. gloeosporioides*.

The virulent fungus has been found to be a Gloeosporium, that is, no setae are produced in the acervulus. Miss Stoneman (9) and Edgerton (44) have called the value of this distinction into question and have presented evidence which indicates that this is a variable factor. The acervuli instead of being dark as in the common wither tip fungus are at first bright flesh-colored and become only slightly darker with age. On ashened portions of the leaf the common wither tip fungus produces black acervuli. This color is due to the sooty setae and to the dark stroma making up the base of the acervulus. These characters develop typically on dead ashened portions of lime leaves. Under identical conditions the pathogenic organism produces bright flesh-colored acervuli even when the ashened portion of the leaf is so dry as to be brittle.

The basidia of the two fungi correspond very well in measurements and in form, altho in exceptional cases a length as great as 50 μ has been observed for conidiophores of the pathogenic organism. The conidiophores of the common wither tip fungus are described as pale sooty at the base. Those of the pathogenic fungus are hyaline thruout. The conidiophores of the common wither tip fungus arise from a well defined black stroma as has been figured by Saccardo, Rolfs (15), Miss Stoneman (9), and others. Those of the pathogenic organism, on the other hand, arise from a closely interwoven mass of hyaline branching hyphae.

The conidia of the common wither tip fungus have a peculiar very characteristic form. Typically one end is rather bluntly rounded, the other end is blunt, but more or less obliquely truncate, and there is a slight constriction at the middle of the spore. The conidia of the pathogenic fungus have one or both ends more or less pointed with very little tendency toward constriction at the middle. The spores of the common wither tip fungus when taken direct from fresh material are stuffed with a coarsely granular plasma, and show a distinct clear nucleus at the middle. The spores of the pathogenic organism lack

these highly refractive granules, consequently they appear at first sight homogeneous. Closer examination, however, discloses the presence of a clear nucleus in the middle, but it does not stand out conspicuously on account of the clear hyaline nature of the rest of the spore contents. The measurements of typical spores of the two fungi are the same, but those of the pathogenic organism vary much more widely from the typical size and form than do those of the common wither tip fungus. These differences of the conidia are sufficient to enable anyone familiar with the two forms to distinguish them if he has absolutely fresh material of both species at his disposal. There is, however, a close enough similarity to account for the previous confusion of the two fungi.

The color of the acervulus, together with the structural differences which it indicates, the absence of setae, and the distinct character of the conidia constitute the salient morphological differences between the two fungi. This distinction of two fungi as concerned in wither tip probably explains the morphological differences which Rolfs (15) noted on the lime and lemon.

It has been found impossible to identify the pathogenic organism with any thus far described as occurring on citrus species. The comparison has not been as satisfactory as would be desirable, due in part to the inadequateness of many of the descriptions, in part to the fact that type material was not available for any comparisons.

The names of the species of Gloeosporium with which descriptive comparisons have been made are given herewith for reference :

Gl. Hesperidearum Catt., *Gl. depressum* Penz., *Gl. Hendersonii* B. et Br., *Gl. Aurantiorum* West., *Gl. intermedium* Sacc. and var. *subramulosum* Sacc.. *Gl. sphaerelloides* var. *majus* Penz., *Gl. Citri* Cooke et Mass., *Gl. Spegazzinii* Sacc., *Gl. citricolum* Cooke et Mass., *Gl. hysterioides* Ell. et Ev., *Gl. tenuisporium* McAlp., *Gl. intermixtum* McAlp. Reference was made for the most part to the descriptions as given in Saccardo's Sylloge Fungorum, but in a few cases the original articles were consulted.

Two of these fungi have thrown us somewhat into doubt, and consequently their descriptions are given herewith in full for comparison with that of the pathogenic fungus with which we are concerned.

Gloeosporium Hesperidearum Catt. Sacc. F. It. t. 1186. Mic. Agrumi. p. 12.—Acervulis gregariis, numerosis, sparsis in vasta macula arida foliorum vel in foliis emortuis, erumpentibus; basidiis obsoletis, fasciculatis, cylindricis, continuis, simplicibus, hyalinis vel basi dilute fuligineis, 8–12 x 4–5; conidiis cylindricis, utrinque rotundatis, eguttulatis, hyalinis, 14–18 x 5–6.5.

Hab. in foliis languidis vel emortuis Citrorum, Messina, Siciliae (Borzi), S. Terenzo (Gibelli).

Gloeosporium Spegazzinii Sacc., Gl. citricolum Speg. F. Puigg. n. 443 (1889) nec C. et M.—Maculis amphigenis, maximis, indeterminatis, albo-cinerescentibus; acervulis amphigenis, laxe gergariis, lenticularibus, minutis, 200-300 diam., fuscescentibus; conidiis cylindraceis, hyalinis, 14–18 x 6–7, utrinque obtusissime rotundatis; basidiis 20–30 x 5, sursum tenuato-truncatis basi fuscellis.

Hab. ad folia viva Citri limonum in hortis prope Apiahy Brasiliae.

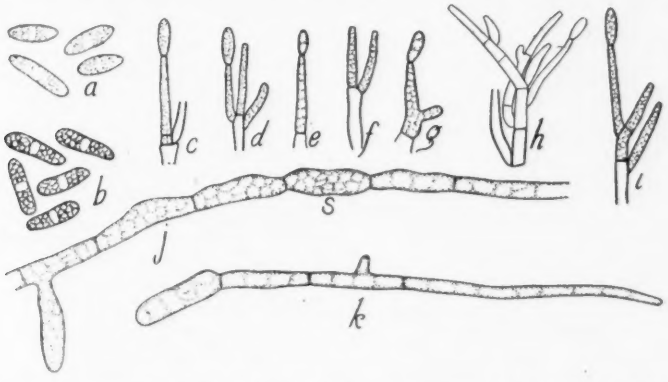

FIGURE 1. *a*, spores of *Gl. Limetticolum*; *b*, spores of *C. gloeosporioides*; *c* to *i*, conidiophores of *Gl. Limetticolum*; and *j*, *k*, germination figures of *Gl. Limetticolum*, the spore at *s*.

The pathogenic species with which we are concerned differs markedly in some respects from even the two described above. No extended discussion of such differences is given here, since it would not be based on a comparative morphological study as has been the case in our observations of the two fungi considered in detail. The physiological and morphological differences which have been described seem sufficient to distinguish the fungus specifically. Accordingly, the name *Gloeosporium Limetticolum* is proposed for it in reference to its lime-inhabiting nature, and the following technical diagnosis, based on a study of material from plants inoculated with pure cultures of the fungus, is given:

Gloeosporium Limetticolum sp. nov.

Gl. maculis plurime in marginibus foliorum, junioribus badiis, mox latericis, maturitate dilute melleis, sed marginibus maculorum adultorum non numquam persistente latericis, magnis, folia juvenia aut ramulos deformantibus; acervulis sparse gregariis, nunc conflu-

entibus, primo incarnatis demum ferrugineis, 50–100μ et ultra in diam., maxime epiphyllis ; conidiophoris (basidiis) dense fasciculatis, simplicibus, continuis, hyalinis per totam longitudinem, 16–30 x 3–5μ, e mycelio dense intertexto et adparenter numquam e stromate distincto; conidiis acrogenis, oblongo-ellipticis, rectis, ad utram finem plus minusve attenuatis, hyalinis, intus homogeneis (i. e., sine guttis aut granulis), in magnitudine formaque variabilibus, 12–20 x 3.5–6μ ; in medio nutritivo cultum numquam pelliculam stromaticam nigram formans.

Hab. in foliis juvenibus ramulisque Citri medicae var. acidae Hook., in insula Cubensi sponte pathogenicum et in caldariis Californicis in foliis ramulisque C. medicae varr. acidae Hook, C. limettae Risso, et C. limonis L. artificialiter infectis.

Type material has been deposited in the Herbarium of the University of California and at Washington, D. C. The report of pathogenic properties on the sweet lime, *C. medica* var. *limetta*, is according to C. O. Smith who is working at Whittier, Calif. with a strain of the fungus supplied by the writer. The material from Cuba was on sour lime, *C. medica* var. *acida*.

SUMMARY

An investigation of wither tip diseases indicates that at least two fungi are concerned in them, instead of one as has been believed heretofore.

Colletotrichum gloeosporioides gave no positive results of a true wither tip nature in the inoculation work.

Wither tip of the lime was found to be due to a fungus which in artificial infection work on those citrus species studied attacked only forms and varieties of one species, *Citrus medica*.

The morphological differences which Rolfs noted for *C. gloeosporioides* on young lime and lemon twigs, foliage, and fruit were probably due to the presence of this fungus on such material.

The fungus was found to be absolutely distinct from *C. gloeosporioides* in cultural, morphological, and pathogenic characters, and apparently also from any fungus thus far described on any citrus species.

The pathogenic fungus of the lime is given the name, *Gloeosporium Limetticolum*, and a technical diagnosis presented for it.

It is believed that a tenable explanation has been offered for the differences of opinion of various investigators regarding the nature of wither tip of citrus species.

I am especially indebted to Prof. Horne for his unfailing interest in this work and for the suggestions which led to the solution of the

problem here presented. Prof. Setchell kindly prepared the Latin rendition of the technical diagnosis and gave me valuable suggestions concerning the description of the fungus. I am indebted to Mr. C. O. Smith of the Whittier Laboratory for most of the California material used in these investigations and for his kindly interest in the work, and to Prof. H. S. Fawcett for the Florida material. Prof. F. S. Earle, of Herradura, Senor Patricio P. Cardin, Chief of the Department of Vegetable Pathology, Estacion Experimental Agronomica, Santiago de las Vegas, and Mr. H. A. Van Hermann of the Mulgoba Nurseries, Santiago de las Vegas, kindly supplied me with the Cuban material.

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EXPLANATION OF PLATE XXII

FIGS. 1, 3, 4. Infected leaves and tip from the plant shown in Plate I, fig. 2. Photographed two weeks after inoculation. Nearly natural size.

FIG. 2. Type specimen from an infected plant in Experiment XV. Specimen placed in the press forty days after inoculation.

FIGS. 5, 6, 7. Infected lemon leaves from the lemon tree in Experiment XXVII, about fifteen days after inoculation.

FIGS. 8, 9. Agar Petri dish cultures of *Gl. Limetticolum*. The same culture photographed with a white and with a black background in order to bring out the distinctions more clearly.

FIGS. 10, 11. Similar photographs of a parallel culture of *C. gloeosporioides*.

PLATE XXI. **Wither tip on limes**

FIG. 1. The lime tree from Experiment IV twelve days after inoculation, showing the characteristic curled and distorted appearance of the leaves of the tips of the shoots.

FIG. 2. The lime tree from Experiment X ten days after inoculation.

PLATE XXII. **Wither tip disease**

SPORE GERMINATION AND INFECTION WITH PLASMOPARA VITICOLA

C. T. GREGORY

(WITH SEVEN FIGURES IN THE TEXT)

GERMINATION OF THE OOSPORES

For a considerable time after the discovery of *Plasmopara viticola* in France various workers were continually attempting to germinate the oospores but, although numerous theories based on the known methods of oospore germination in other Peronosporales were advanced, no one was able to accomplish this satisfactorily.

Millardet [1] was of the opinion that the oospores germinate by swarmspores. He based this conclusion on the facts brought out by DeBary's studies on Cystopus [3], namely, that the oospores in members of this genus germinate in this manner.

On the other hand, De Bary [2] gives four methods of germination of the oospores in the Peronosporales: first by the production of a germtube which becomes the thallus and which then again produces oospores as did the mother plant; second, by the oospore becoming a sporangium, with its entire content breaking up into zoospores, which, on germination, produce the thallus on which oospores are eventually again borne; third, by the oospore germinating by either of the two preceding methods, according to the conditions; fourth, by the formation of a promycelium, which, in turn, produces a few conidia.

Fréchou [4], Ch. Richou and Viala [5] all claim to have seen the germination by zoospores. Fréchou also has seen the oospores produce a long voluminous tube which might be interpreted as a conidiophore although there were never any conidia produced on it.

On the contrary, Prillieux [6] has only seen and figured the germination by means of a long branched tube which had all the appearances

[1] Millardet, P. M. A. Sur le rôle des spores d'hiver du mildou (P. viticola de By) dans la reinvasion par ce parasite. Mem. Soc. Sci. de Bordeaux. **5**: XXIV-XXVII. 1883.

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of a conidiophore but produced no conidia. From this fact and from his scepticism as to the ability of swarmspores to be disseminated to the leaves of the vine in the spring, he comes to the conclusion that the oospores must produce conidia on conidiophores in nature but that some favoring condition, possibly the leaf tissue, was lacking in his experiments. Previous to this time Cornu[7] had already evolved the theory that the oospores produce conidia. His conclusions were largely based on his work with the family Saprolegniales.

Out of this contradictory mass of evidence one might derive the the conclusion that the oospores germinate by zoospores most commonly and that conidia are rarely produced, but in the mind of the writer it seems that no satisfactory evidence had been presented in proof of either view of the matter, and in an effort to obtain some definite evidence the following investigation was undertaken.

During the autumn of 1911 a large number of mildewed leaves were collected from both Niagara and Delaware vines, care being taken to obtain those having old and extensive dead areas. In this way the chances of obtaining oospore material would be greatest, since the fungus would have sufficient time to produce them in abundance, which might not be the case with small spots of the late infections. These leaves were enclosed in a wire-screen case to prevent them from blowing about and were placed on the ground to winter over under as natural conditions as possible.

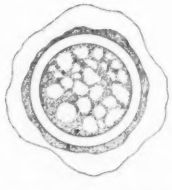

FIG. 1. Mature oospore of Plasmopara viticola

In February of 1912 a number of leaves from the case were brought to the laboratory and placed in petri dishes on moist sand. In doing this the early spring conditions of abundant moisture were imitated, so that after a few weeks time the leaves were in a very soft and partially rotted condition. In March bits of these rotted leaves were examined for oospores. It was merely necessary to tease the leaf tissue apart in order to find an abundance of brown oospores lying singly, or in groups, in the tissue or free in the water. These brown oospores (Fig. 1) have a thick, smooth endosporium overlaid by a

[7] Cornu, M. Étude sur les Peronosporées. II. Peronospora des vignes. Paris, **1882** : 1–91. *Pls.* 1–4. [See Mycol. **3** : 95.]

thinner rough exosporium and lie free in the oogonial cavity. The oogonial wall collapses about the oospore in a very wrinkled and ridged condition. The bits of leaves and oospores were placed on slides with as little water as possible, or on squares of moist blotting paper, so as to insure abundant aeration. Over twenty-five preparations were

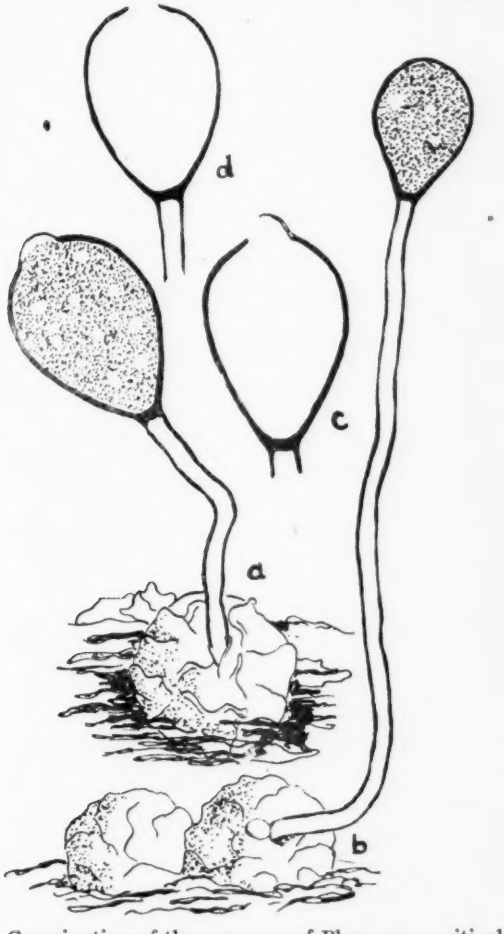

FIG. 2. Germination of the oospores of Plasmopara viticola.

a. With a mature conidium. *b*. With an immature one. *c*. An unusnal type of germination. *d*. Germination of conidium shown in *a*.

made in this way and kept in moist chambers at about 20° C. From time to time examinations were made of the oospores to note any changes which might occur in the contents, or otherwise.

On March 29 the first germination was observed. On this date a few bodies having the exact structure and appearance of the conidia

of *Plasmopara viticola* were found. These conidia were borne aloft on stalks or promycelia of varying lengths and could be observed to proceed, in nearly every case, from one of the brown oospores (Fig. 2). In certain cases it was not possible to see the germinating oospore, owing to the thickness of the leaf tissue in which it was imbedded. After this date the germinating oospores were found almost daily until about April 16. Up till the latter part of May other cases were found but no accurate record was kept of their appearance as had been done at first.

From a small crack in the oospore wall the promycelium grows out through the oogonial wall and at its extremity there is produced an ovate conidium which is cut off by a septum (Fig. 2 a). The conidium has at the distal end the thin-walled projection or papilla characteristic of the conidia of *Plasmopara viticola*. In size these conidia vary from 31 to 47 μ long by 27 μ wide. While the average summer conidia are a little smaller in size there are, nevertheless, certain ones of them which are quite as large as those produced from the oospores. Usually there is only one promycelium, with its accompanying conidium, from an oospore but occasionally two may be produced. In this case the conidia are smaller than when produced singly. It seems certain that the oospores germinate very rapidly since the earliest stages of the promycelium have never been observed, though at times immature conidia have been found (Fig. 1 b). In no case has the germination of the oospore by swarmspores been observed.

The germination of these conidia has never been followed, but the empty shell (Fig. 2 c and d) has been found, thus indicating that the germination follows the usual method by swarmspores. It is also quite certain that the conidia are easily broken loose from the promycelium since in trying to arrange some specimens in better position for observation the bits of leaves were very slightly moved and the conidia were broken away and lost.

Thus it seems that the oospores in the leaves on the ground produce their conidia on promycelia, and that the winds and rains break these loose and blow or spatter them to the leaves where they germinate and produce the swarmspores, which in turn infect the leaves. That the rains could very easily scatter the spores to the leaves can be seen by examining the lower leaves of vines on well-cultivated ground after a rather heavy rain, when mud will be found on both the upper and lower surfaces of the leaves spattered to the height of two or three feet. This fact, however, would serve equally well to explain distribution of the swarmspores were the germination of the oospores by this method.

GERMINATION OF CONIDIA

Historical. Dr. Farlow[8] was the first to describe the germination of the conidia. He states that germination occurs in about an hour and a quarter and that from two to seventeen swarmspores are produced. He mentions a pause of the mass of swarmspores at the mouth of the conidium, followed by the jerking apart of the individuals. The swarmspores also constantly change in shape while swimming about. The swarming lasts for fifteen to twenty minutes, after which the swarmspores come to rest, round up, drop their cilia and in about a quarter of an hour produce a germ-tube. Occasionally they do not germinate in this given time but require a much longer period. Ordinarily the germination is quite regular. Both Cornu and Scribner[9] quote Dr. Farlow's statements concerning the germination of the conidia.

Patrigeon[10] states that water, and not simply damp air, is necessary for the germination and that a temperature of 25° to 30° C is best. He mentions, as another possible method of germination, the emission of the entire contents of the conidium as a naked mass of protoplasm which rounds up and produces a germ-tube, though he acknowledges that this is a very exceptional method.

In the same year, Cuboni[11] claims that intense light prevents the germination of the conidia.

Wüthrich[12] claims that the conidia germinate in one to two hours and that the zoospores swarm for three to five hours and after fifteen to twenty hours germinate. He never observed the germination of the conidia by a germ-tube.

Viala[5] assembles all of the important contributions on the mildew up to 1893 and also adds a few points brought out by his own investigations. He figures and describes three methods of conidial germination. In the first, germination by zoospores is said to occur within the space of one-half to one hour at a temperature of 28° to 30° C., five to eight irregular biciliate swarmspores being produced. These cilia are

[8] Farlow, W. G. On the American grape vine mildew. Bul. Bussey Inst. **1**: 415–425. *pls.* 2–3. 1876.

[9] Scribner, F. L. Report on the fungus diseases of the grape vine. U. S. Dept. Agr., Div. Bot., Bul. 11: 10–15. *Figs.* 1–2. 1886.

[10] Patrigeon, G. Le Mildiou. pp. 16–25. *Figs.* 1–10. 1881.

[11] Cuboni, J. La Peronospora dei grappoli. Atti Congr. Nazion. Bot. Critt. in Parma. **1887**: 91–108. [French translation by A. Picaud. 1889].

[12] Wüthrich, Ernst. Ueber die Einwirkung von Metalsalzen und Säueren auf die Kaumfähigkeit der Sporen einiger parasitischer Pilze. Zeitschr. Pflanzenkr. **2**: 16–31. 81–94. 1892.

figured, after Millardet, as having a small swelling or lobe at the distal end of each. In the second method, at a temperature below 20° C., the conidia may exude their entire contents, which round up, and become surrounded by a membrane, germinating by a germ-tube. This method is said to be very rare. A third and much more rare method of germination has been noted by Viala to occur with conidia taken from conidiophores which had become a dull white due to desiccation. In this case the conidium produced a long tube directly. This tube was emitted through the distal end, the point where the wall is thinnest and through which the swarmspores always escape. These three methods of germination have been reported by deBary in numerous other of the Peronosporales so it is reasonable to suppose that they might occur in this species.

Prillieux's[13] report of the germination of the conidia is not materially different from those of the numerous other workers. Thus, since the work of Farlow, Millardet, Prillieux and a few others, the germination of the conidia has been left entirely unstudied until very recently.

Conidial Germination. When one examines a mass of the conidiophores taken from a leaf usually three types of conidia can be distinguished. Some are finely granular and hyaline, others are densely and coarsely granular and more or less brown, while others are partly or wholly filled with a highly refractive drop which appears to be oleaginous in nature. Any of these types will germinate except the old coarsely granular type, which, so far as I have been able to determine, does not do so.

Ordinarily germination is brought about by placing the conidia in a suspension of water in a Van Tieghem cell or in a drop of water on a slide. At times, however, the conidia can not be germinated under these or any other conditions, so far as I have been able to determine. At best the germination is rather uncertain.

The conidia are ovate and are attached to the conidiophore at the small end. At this end the wall is relatively thick but it grows gradually thinner toward the apex. At the tip of the conidium there is a papilla or cap-like structure which is very much thinner and is also more hyaline than the remainder of the wall. The papilla is variable in prominence. Sometimes it is almost lacking and at other times so pronounced that it appears like a cap which might be pushed off by swarmspores at germination. It is at this point that the conidial wall disappears, leaving an opening just the size of the papilla, through which the swarmspores escape.

[13] Prillieux, Ed. Maladies des plantes agricoles **1** : 97–131. 1895.

At first the protoplasm is finely granular (Fig. 3 a) but about an hour after being placed in water there appear lighter hyaline spots in the protoplasm which at the same time becomes a little denser and more granular (Fig. 3 b). This continues until there is a dense granular mass with clear, distinct spots arranged at regular intervals. In a short time there appear in the protoplasm dark lines which mark out portions about each hyaline spot (Fig. 3 c). These lines become more and more distinct and finally there are slight indentations along the margin of the previously smooth protoplasm (Fig. 3 d). The content

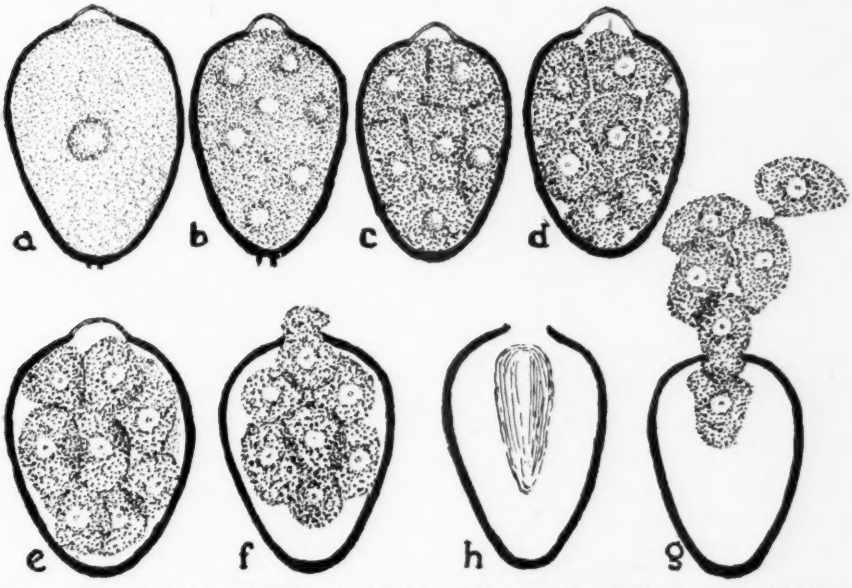

FIG. 3. Various stages in the germination of the conidia of Plasmopara viticola. *h*. The appearance after germination of a conidium containing an "oil drop"

of the conidium is now very rough and irregular. By focusing carefully the individual swarmspores can be made out. In a few minutes the spores break apart and become distinct bodies (Fig. 3 e). At this point there is a pause during which it seems that the spores must burst forth immediately and, coincidentally, there seems to be a slight movement among them. Suddenly through the tip of the conidium there appears a bit of protoplasm of one of the swarmspores (Fig. 3 f) which slowly forces its way out until the opening becomes maximum in size when the entire mass of spores, jerkily but rapidly, slips out. It is quite certain that the opening is at the papilla, and is probably brought about by the dissolution of the wall at this point and not by its breaking, since no remnant of the wall can be found after evacuation.

The swarmspores remain for an instant at the end of the conidium and then pull apart and swim away (Fig. 3 g). It is quite probable that the flagella are formed at this time by the pulling apart of the spores. There is a considerable jerking and wrenching before they separate. At times two spores remain attached for a long time and, finally, by dint of much pulling they snap apart and swim away. At other times as many as four or five spores are joined together by their flagella (Fig. 4 a). Thus it would seem that the flagella are slender threads of protoplasm pulled out from the spores as they split apart.

Sometimes all the swarmspores do not escape from the conidium at once, but one or two remain swimming about within. These spores experience considerable difficulty, so to speak, in escaping, seeming to be unable to squeeze their nuclei through the opening. When they do manage to escape they usually are constricted at the center into a dumb-bell shape.

The number of spores produced varies greatly. Some of the large conidia produce as many as 15 to 17, while some small ones have only 1 or 2. The normal number is 5 to 8.

The conidia containing the oil drop germinate readily at times. In this case the oil drop is left behind in the conidium (Fig. 3 h), the entire mass of protoplasm going to make up the swarmspores.

Swarmspores. The shape of the swarmspores is plano-convex with a keel or ridge along the flat side. In the center near the flat surface there is a light hyaline spot, the nucleus. Near it, and less brilliant, is another spot which by tinting with stains appears to be a vacuole. From two points each side of the nucleus arise the flagella, which are of unequal length. This is the normal shape of the spores but there are many exceptional shapes produced at times. It is quite apparent that when the spores are newly formed and escaping from the conidium they are very plastic and capable of taking any form, but once they are fixed in any given shape it is difficult for them to change. Thus, if in pulling apart the spore becomes pyriform or some irregular shape it remains so.

It has been stated by some authors that the swarmspores are constantly changing their shape when swimming about. It appears to the writer, however, that there is no actual change, the apparent change being due to the rolling motion of the spores as they swim. This, combined with their peculiar shape, produces the illusion.

The size of the swarmspores varies, being 6–7 x 7.5–9μ. The flagella are from 27 to 33 μ in length.

After swimming about for approximately one-half hour the swarmspores gradually come to rest, round up and surround themselves with

a thin membrane (Fig. 4 b). They apparently do not immediately "drop" their flagella but, rather, absorb them, because, after becoming globose, they continue to whirl and move back and forth until they gradually come to rest. They would probably stop abruptly if the flagella were immediately dropped. Staining which has clearly revealed the flagella on the motile forms has failed to reveal them lying free among the quiescent forms in the same preparation. These remain globose for fifteen minutes, more or less, and then there appears a slight protuberance (Fig. 4 c) from one side, accompanied by the appearance of a vacuole at the opposite side. This is the germ-tube. It elongates and the contents of the spore follow. This continues until the contents have passed out into the tube, leaving the thin wall. After the tube reaches a certain length there may be produced an appressorium (Fig. 4 c) from which another tube arises later.

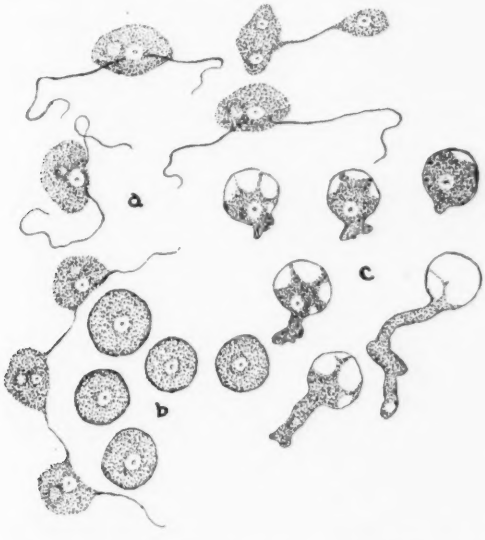

FIG. 4. *a*. Swarmspores. *b*. Quiescent stage. *c*. Germination of swarmspores, the one in the lower right corner showing an appressorium

The time occupied in these various processes is quite variable and no definite limits can be given. Usually the first swarmspores appear in about one hour to an hour and a half but they continue to appear in other conidia for about five hours. The rounding up begins in about an hour and a half to two hours after expulsion. Complete germination may occupy about three hours or it may require as much as twelve hours.

INFECTION

Recent work with the mildew has concerned itself with a study of the method of entrance of the germ-tube of the swarmspore into the leaf. The older workers have taken it for granted that, since the greater proportion of the conidia must fall on the upper side of the leaf (a conclusion based on experiments of Millardet[14]), the tube must penetrate the epidermis of the leaf directly. This view is not surprising, since De Bary found that in a number of the Peronosporales the germ-tubes do penetrate the epidermis directly.

Dr. Farlow noted that infection took place much easier on the lower than on the upper surface of the leaves but he only accounts for this by the fact that the lower surface is more concave and holds the water much better than the upper. He states that he has produced infection on the upper surface of the leaf in numerous cases.

In 1908 Ruhland and von Faber[15] found that infections do not occur on the upper surface. Immediately following this work a large number of investigators commenced experiments which completely verified the pioneer experiments of these authors.

Müller-Thurgau[16] in Switzerland was the first to publish extensively on this subject. He gives very complete and numerous experiments, all of which prove that infections occur only on the lower surface. He has seen the penetration of the germ-tube through the stomates and found the mycelium in the sub-stomatal cavity and even found a few of the cells about the cavity browned by the effects of the fungus. He also states that three hours is sufficient for the germination of the swarmspores and penetration of the stomates, while after thirty six hours the destruction of the cells is noticeable. He had difficulty in infecting very young leaves, and explains this, according to Dr. Faes, by the fact that the stomates have not yet opened. Hail wounds and other injuries to either surface may also serve as a court of infection.

Faes[17], very shortly after, gave an account of some infection experiments which completely verify these conclusions. Although he did not see the penetration of the germ-tube into the leaf he did note

[14] Millardet, P. M. A. Observations nouvelles sur la developpement et le traitement du mildiou. Jour. Agr. Prat. 2. **1886** : 663-667. 1886.

[15] Ruhland, W. and von Faber, F. C. "The biology of Plasmopara viticola." Ber. Tatigk. Kais. Biol. Anst. Land und Forstw. **1908** : 19. [Abstr. in Exp. Sta. Rec. **23** :251]. Original not consulted.

[16] Müller-Thurgau, H. Infektion der Weinrebe durch Plasmopara viticola. Centbl. Bakt. u. Parasitenk. Abt. 2, **29** : 683-695. 1911.

[17] Faes, H. Nouvelles recherches sur le developpement et le traitement du mildiou. Rev. Vit. **36** : 489-493. 517-524. 545-550. 1911.

the assembling of the swarmspores about the stomates. Istvánffi and Palinkas[18] also made a number of infections on leaves under natural conditions, with the same results.

Ravaz and Verge[19], later in the same year (1911), acknowledge, as a result of tests made by them, that infections undoubtedly occur through the stomates, but in view of the fact that spraying on the upper surfaces of the leaves has in years past controlled the disease, and in the belief that the greater number of conidia must fall on the upper surface, they are convinced that if inoculations were made on the upper surface in a film of water which extended around the edge of the leaf, infections might be produced by the swarmspores swim-

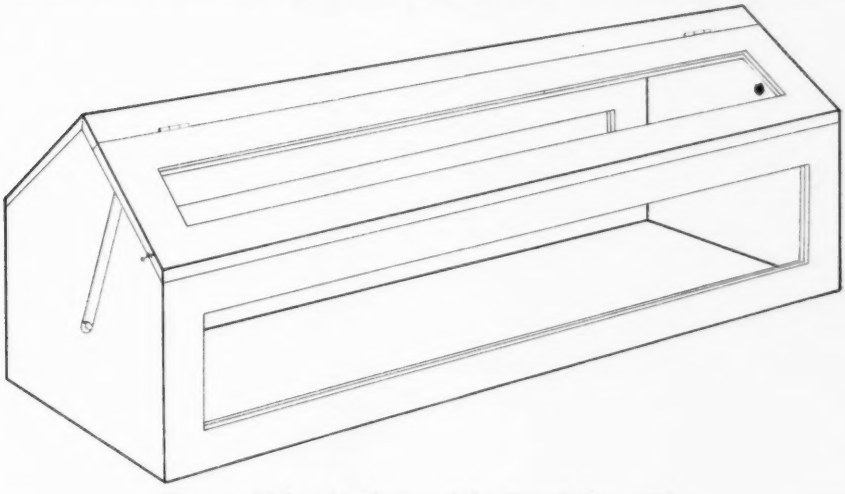

FIG. 5. Moist chamber used for inoculation work

ming over the edge and thus to the stomates. In support of this claim they state that in all of the experiments, inoculations on the upper surface were made in small isolated drops which gave the swarmspores no access to the lower surface. In addition they found that the swarmspores were able to swim for a distance of at least 46 millimeters.

In order to verify this work on American varieties the writer conducted the following experiments during July and August of the summer of 1912 at Esperanza vineyard in the Keuka Lake district of New York. From observations made during the summer it was

[18] Istvánffi, Gy. von and Palinkas, Gy. Infectionsversuche mit Peronospora. Centbl. Bakt. u. Parasitenk., Abt. 2, **32**: 551–564. 1912.

[19] Ravaz, L. and Verge, G. Sur le mode de contamination des feuilles de vigne par le Plasmopara viticola. Compt. Rend. Acad. Sci. (Paris) **153**: 1502–1504. 1911.

found that the mildew attacks all the tender green parts of the vine, namely, the leaves, stems, tendrils and fruit. In severe cases there is produced a swelling of the stem, while the fruit assumes a peculiar livid color and becomes hard, followed by a reddening, or browning, and shriveling of the berry. On the leaves there are yellowish spots produced at first, followed by a red-brown color and drying of the tissues. The size of the spots depends on the weather conditions and the age of the leaves, being largest on young leaves or during wet weather.

For this work the susceptible variety, Delaware, was selected. The leaf of the Delaware is also relatively smooth, that is, free from the hairs which so abundantly clothe the lower surface of most of the American varieties.

An attempt was made to conserve the natural conditions as much as possible during the experiment. To this end a glass infection chamber was made of the shape shown in figure 5. The sloping hinged top was designed to shed rain and also to afford a ready means of opening the chamber.

A branch was freed from the wires and introduced into the chamber through a slot cut at one end. The branch was so arranged that the leaves all lay lower side up, for inoculations on the lower surface, or in the natural position, when the inoculations were made on the upper surface. At each end of the chamber were placed dishes of water each containing a sponge, thus increasing the evaporating surface. In this way the air of the chamber was kept moist and single drops of water could be held for 12 to 24 hours. The whole chamber was shaded with white cloth to reduce the intense heating of the sun.

Before making inoculations a ring of India ink was always made on the leaf and into this a drop of rainwater or distilled water was placed. Conidia were taken from leaves which had been kept in a moist chamber over night, so as to allow the abundant production of fresh spores, and placed in the drop.

The chamber was usually examined once a day at least to be sure that the drops did not evaporate, and in some cases even this precaution did not prevent the drying of the drops especially during the hot weather of July.

The results are shown in the table on the next page.

The percentage of infection on the lower surface was 54.75. The very low percentages in some cases can be explained by the drying of the drops, mentioned before, by the fact that certain of the leaves were too old to be infected and by the peculiar fact that in some cases the conidia could not be germinated abundantly under any of the conditions that were given.

INFECTION EXPERIMENTS

Date	Inoculations		Infections	
[1] June 30	5 leaves, upper surface			0
July 9	7 " " "			0
" 14	6 " " "			0
			Lower	Upper
[2] July 19	16 spots, lower; 4, upper surface		7	0
" 28	18 " "		13	
" 30	33 " "	8 " "	13	0
Aug. 1	29 " "	9 " "	26	0
" 5	40 " "		8	
" 6	55 " "		10	
" 13	60 " "	3 " "	20	0
" 15	58 " "		24	
" 16	57 " "		46	
" 18	50 " "		47	
" 19	38 " "		34	
" 23		37 " "		0
" 24		40 " "		0
" 28		36 " "		0
" 31		38 " "		0
Totals	454	175	248	0

So far as could be determined infections occurred at any place on the lower surface. The inoculations on the upper surface were, however, made only along the main veins and the margin of the leaf.

In order to see the penetration of the germ-tube into the tissue conidia were placed in a drop of water on the lower surface of freshly plucked leaves which were placed in a moist chamber. These were allowed to stand for about twelve hours to insure the complete germination of the conidia and the penetration of the germ-tube into the leaf. Following this, a drop of eosin was placed in each conidium-laden drop and allowed to act for 10 to 30 minutes. At the end of this time the stain was removed carefully with blotting paper and replaced with water. This method gives deep red spores which stand out sharply against the green of the leaf.

It is now only necessary to cover the bit of the leaf with a cover glass and examine the surface with the 4 mm. objective of the microscope in order to see the red swarmspores abundantly scattered over the surface of the leaf and in numerous cases producing germ-tubes of varying lengths. These tubes invariably are directed towards the stomates and, when long enough, can be seen to dip downward into

[1] In experiments June 30–July 14 the conidia were spread over the whole surface.

[2] In experiments July 19–Aug. 31 the conidia were placed inside the ink rings, mentioned above.

the opening (Fig. 6). It is not uncommon to see two or three, or even more, swarmspores clustered about a single stomate and all of them sending tubes into the leaf. In cross-section (Fig. 7) the tubes can be seen to extend down into the sub-stomatal cavity to the surrounding cells. The browning of these cells mentioned by Müller-Thurgau has not been observed, probably because the experiments were not long enough continued.

The germ-tube after penetrating the stomate seems to become somewhat larger in diameter as compared with the tubes produced by the spore when germinated on a slide. So far as has been observed, the tubes do not produce in the sub-stomatal cavity of the leaf the appressorium so often seen in the slide cultures. If this body serves as a resting condition of the germ-tube, as may be the case, it would not be needed in the cavity where food is abundant and growth unimpeded.

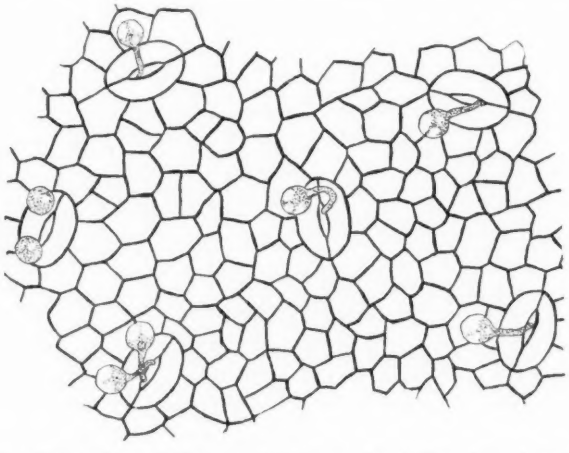

FIG. 6. Clustering of the swarmspores about the stomates and the penetration of the germ-tube. Surface view after 12 hours

It is unquestionably true, as Müller-Thurgau and other investigators in Europe state, that infection of the grape leaves occurs through the stomata of the lower surface of the leaves and that the penetration of the epidermis, though it may be possible, as was formerly supposed, does not commonly occur.

Apparently it has been assumed by many that spores of any wind borne fungus would naturally fall upon the upper surface of the leaves. Considering the extreme lightness of the conidia of this or of most fungi this is quite likely a groundless assumption. It is doubtful whether the smeared glass plates suspended in the vineyard and used by Millardet to determine the lodgment of spores is quite comparable

to the condition offered by the leaf of the grape. The glass plate would remain rigidly horizontal or vertical at all times, whereas a grape leaf is being constantly blown about, even exposing at times the lower surface and, moreover, it is constantly rubbing against the upper surface of the leaf below. Thus, if conidia lodged on the upper surface of any leaf they might be easily transferred to the lower surface of the leaf next above.

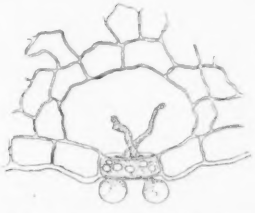

FIG. 7. Penetration of the germ-tube through the stomate into the substomatal cavity. After 12 hours

If one examines the leaves during a moderately heavy rain in summer he will find that the lower surface is as wet as the upper. This wetting is due largely to the spattering of the water from the upper surface of the leaves next below. Thus, the conidia could be easily carried in this way with the spattering drops of water.

A final possibility, is that suggested by Ravaz and Verge, namely, that the conidia may fall on the upper surface, where they germinate and the swarmspores may then swim over the edge of the leaf to the lower surface. As far as the film of water about the leaf is concerned this method of infection is quite possible.

In conclusion, the writer wishes to acknowledge his indebtedness to Dr. Donald Reddick for numerous helpful criticisms and suggestions.

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DOES PHYTOPHTHORA INFESTANS CAUSE TOMATO BLIGHT?[1]

HOWARD S. REED

The tomato crop in the southwestern part of Virginia has suffered for a number of years from a blight caused by Phytophthora. The disease usually makes its appearance about the time the vines begin to ripen the fruit. From that time on until they are killed by frost the blight becomes worse, the fungus attacking the leaves, stems and fruit. The leaves turn black and wilt, giving an appearance similar to that of a vine which has been killed by frost. The fungus attacks the fruit soon after it appears on the leaves, causing a rather characteristic rot which is very destructive. The infection appears to take place underneath the calyx and, as a result of this, the fruit begins to rot around the stem-end.

In 1911 the writer published a brief account of this and another tomato disease as Bulletin 192 of the Virginia Experiment Station. At that time he was not certain that the fungus identified as Phytophthora was the same as that found on potatoes and known as *P. infestans*. It had been assumed by previous workers that the two were identical but direct proof did not appear in the literature. It was thought best in the publication cited to refrain from placing the tomato fungus under *P. infestans* because observations failed to show any considerable amount of potato blight in southwestern Virginia. In many instances tomatoes and potatoes were grown near each other. The tomatoes showed large amounts of blight while the potatoes showed very little, hence the suggestion was made that the blights might be caused by varieties of *P. infestans*. In view of these facts, it was deemed wise to await the results of further work before deciding definitely where to place the fungus.

The publication of these views in the bulletin above mentioned called out several very interesting letters from plant pathologists who had had extensive experience with the potato blight in the northern states. From their letters it appears that in New York and the New England states, where Phytophthora is a destructive disease on potatoes, they find little if any injury due to the action of this fungus upon tomatoes.

Experiments were carried out in 1911 and again in 1912 in which potatoes and tomatoes were planted in alternating hills. The plants when half grown came into contact with each other and thus afforded a very favorable opportunity for the disease to spread from one plant to another. The experiment in 1911 failed to give any results be-

[1] Paper No. 20, from the Laboratory of Plant Pathology, Virginia Agr. Exp. Station, Blacksburg, Va.

cause the Phytophthora did not appear in Virginia that season on either of its host plants. In 1912, however, the fungus reappeared and it was possible to determine its relationship.

In 1912 the Phytophthora blight was first found on August 12th in the unsprayed portion of the interplanted plat (potatoes and tomatoes). On the same date it was also found in the lime-sulphur sprayed plat (tomatoes only) which adjoined the potatoes. From that time on, the disease spread on both the potatoes and tomatoes.

It was found possible to infect potatoes and tomatoes with the blight by taking spores from either host plant. When examined in the laboratory no difference was noted between the spores taken from the two sources. It has been noted each year that the disease has not appeared until the advent of weather cool enough to bring lower temperatures during the night. At the altitude of Blacksburg (2200 feet) the cool nights bring an abundant formation of dew which apparently gives proper conditions for the germination of the swarm spores of the fungus. In localities in southwestern Virginia at higher altitudes the disease appears earlier and causes correspondingly greater injury. This relation of the disease to altitude is quite striking for the disease is practically unknown in this state below about 2000 feet, but is universally present at altitudes greater than that. The reason would appear to be connected with the cooler temperature and the more abundant formation of dew. During the last few years the higher portions of the state have had more rain during the summer months than the lowlands and this undoubtedly also acts as a predisposing factor.

Reverting now to the question of the apparent immunity of the potato crop, it may be said that the period of active growth for the potato is practically over at the time the Phytophthora blight makes its appearance. Potato growers have found that it was more profitable in this region to plant at the earliest possible date. We found, on careful inspection, that the vines of the later-planted potatoes showed the presence of more or less Phytophthora blight. If the main potato crop were a late one there is now no reason to believe that it would escape the attack.

Some attention has been given to the method by which the blight fungus may live over the winter. There is some evidence that it may be transmitted in tomato seeds, for it is quite easy to find fungus mycelium in the seeds of the infected fruit. It has not been shown, however, that the mycelium retains its vitality during the wiuter. At present it seems more probable that the present epidemics of tomato blight start from the potato, since the early infections are usually on tomatoes which grow near potato vines. In making an inspection of

the diseased areas it was found that the tomato blight was always more severe in the vicinity of potato vines. We are led to conclude, therefore, that the Phytophthora of the tomato is identical with that of the potato and that the apparent immunity of the latter is due to the practice of the farmers in growing comparatively early crops. The reason for the apparent immunity of the tomato in the northern states is not yet evident. It would be interesting to follow this question further in one of the localities farther north, where the potato blight is the more serious disease.

The effect of the fungus upon subsequent generations of the host plant was observed through two generations. Seeds were collected in the fall of 1910 from both green and ripe fruits infected with the disease. These seeds were planted in the green house and the plants grown to maturity. The seed from the immature green fruit gave much better plants. All seed taken from ripe infected fruit gave abnormal plants which showed more or less chlorosis and produced small, bitter fruit. No Phytophthora appeared upon either set of tomatoes grown in the green house ; in fact, the disease has seldom shown itself in the Station greenhouse.

Seed was collected from both sets of plants and a second crop of plants raised. These were transplanted to the field and formed a part of the field experiments of 1911. Here, again, the plants descended from the diseased ripe fruits were inferior to those from the diseased green ones. The former were early attacked by *Septoria lycopersici* and the foliage severely injured. As stated above, no Phytophthora developed in 1911 on any of the fields in this part of the state. Had Phytophthora been present, there is reason to expect that the injury would have been more severe on the plants descended from the diseased ripe fruits. These facts lead one to think that the Phytophthora disease is not transmitted by the tomato seed, but that the infection of the seeds may produce a weakened condition which is manifested by the production of inferior offspring.

Bancroft[1] reports a fruit rot of tomato which he believes to be caused by *Phytophthora omnivora*. He reported that the brown seeds of the infected plants were capable of germination but produced plants which bore infected fruit. With this our results disagree.

Experiments upon remedial methods have been carried on for three years and are to be further continued. Up to the present time the greatest success has been obtained from the use of bordeaux mixture as a spray. Varieties like Red Cherry and Red Pear display almost complete immunity to this blight but to date no successful hybrids have been produced. Among the larger-fruited varieties Red Rock and Buckeye State have shown the greatest resistance.

VIRGINIA AGRICULTURAL EXPERIMENT STATION,
BLACKSBURG, VA.

[1] Jour. Bd. Agr. (London) **16** : 1012. 1910.

APPLE RUST CONTROLLABLE BY SPRAYING

E. T. BARTHOLOMEW

Apparently there has been a rapid increase in recent years in loss to apple orchardists from the cedar rust, *Gymnosporangium juniperae-virginianae* Schw. Experiments show that in many cases the simplest and best control measure is the destruction of the red cedar (*Juniperus virginiana*) for a considerable radius about the orchard. That this method is not always practicable becomes evident if we consider conditions that exist in various localities. For example, certain orchards are located in valleys surrounded by cliffs or steep hillsides covered with cedars; the cedars are valuable for posts, poles, etc.; they are often used for ornamental plantings; or hedges of these trees may be used to protect farm buildings and even the orchards. Under such circumstances the small orchardist usually prefers to sacrifice his susceptible apple trees rather than destroy the cedars.

General orchard observations and artificial inoculations show that certain varieties of apples are more susceptible to the rust than others. In Iowa (2) the Hibernal, Oldenburg, Fameuse and Canada Baldwin are said to be resistant, while the Wealthy suffers most. In Nebraska (4) the Duchess, Sweet June, Yellow Transparent, Ben Davis, Maiden Blush, Winesap and Grimes' Golden are least affected, and the Wealthy, Red June, Whitney, Jonathan and Missouri Pippin are most readily attacked. Reports from Virginia (6) show the Winesap, Mammoth Black Twig, Northwestern Greening and Albemarle Pippin to be the most resistant and the York Imperial, Brier's Red, Jonathan and Rome Beauty to be the most susceptible of the principal commercial varieties. In this state,[1] the Wealthy and Peerless Sweet take the lead as to susceptibility. The Jonathan, Whitney and Sweet Wine are more resistant, while the Wolf River, Northwestern Greening, Fameuse, Duchess, McMahon and McIntosh are very slightly, if at all, affected. Lists might be given for other states, but these are sufficient to show that each section of the country has its own problem, for the variety best suited to one locality may be poorly adapted to another.

[1] These conclusions as to Wisconsin conditions are based primarily on records made by L. R. Jones at Lake Mills, Wis., where there is a large collection of varieties closely aggregated, supplemented by data from Frederick Cranefield, Secy., Wis. Hort. Soc., and our own observations at Baraboo.

This marked difference in varietal resistance shows clearly the importance of planting non-susceptible varieties as a second means of keeping the rust out of the orchards. However, this, like the removal of cedars, is not always practicable, for very often varieties that are most resistant to the rust may be more susceptible to other diseases, or may take low rank as to vigor or productiveness. Unfortunately, in Wisconsin the Wealthy, which is the most susceptible of all to rust, has been one of the favorite varieties for planting.

The two preceding methods are not always desirable and experiments have been carried on in Vermont (1), Iowa (2), Nebraska (3, 4) West Virginia (5), Virginia (6), and other places attempting to control the disease by spraying either the cedars or the apple trees, or both, but these experiments have been attended with failure or only partial success. Probably the most favorable results were obtained by N. J. Giddings (5), who reported one such case of control.

The apple rust having become especially destructive in Wisconsin during the past three years, the writer, under the direction of Professor L. R. Jones, and with the assistance of Messrs. I. E. Melhus and A. G. Johnson, made an attempt during the past spring to control the disease by spraying. The results, though obtained from experiments extending over only one season, were such that it seems justifiable to publish at least a preliminary statement of them.

The place chosen to carry on the experiment was near Baraboo, Wisconsin. The Wealthy being most susceptible, trees of this variety were selected. They were chosen in three different orchards. In the two which were nearest the source of infection Wealthy trees died during the past summer. This was due to their being almost wholly defoliated during the two previous seasons by the ravages of the rust. One of the orchards is located directly at the base of a bluff covered with cedars; the second and third orchards are located about a quarter of a mile and a half mile, respectively, from the same bluff.

Very close watch was kept on weather conditions, the development of the apple foliage and on the maturing of the cedar galls. Immediately following the formation on the cedar galls of the jelly-like telial extrusions and sporidia the apple trees were sprayed with Bordeaux mixture, 4–4–50 and 3–3–50. This was done before sufficient time had elapsed for the transfer of the sporida from the galls to the apple foliage. The first spraying was on May 15, with subsequent applications May 22 and May 30. The proper time for spraying, however, cannot be designated by any fixed dates, for *the crucial time for action depends entirely upon such weather conditions as favor the development of the cedar galls.* Collections of cedar galls were made at the time of

each spraying and these show that on May 15 there were only a comparatively few extrusions, while on May 22 probably half of the gall was covered; not till the time of the third spraying (May 30) was the gall completely covered with the gelatinous masses. This shows that the period of possible infection probably extends over an interval of some three weeks.

During the latter part of the summer, after the rust had fully developed on the apple foliage, a limb was collected indiscriminately from each of the sprayed and unsprayed trees and the number of infected and uninfected leaves was determined, together with the minimum and maximum number of infection spots on the diseased ones. The results are shown in the following tables:

TABLE 1. AMOUNT OF INFECTION ON BRANCHES TAKEN FROM TREES IN ORCHARD AT BASE OF CEDAR BLUFF

Sprayed

Branch from tree number	Number uninfected leaves	Number infected leaves	Percent leaves infected	Number infections on various leaves
1	86	17	16.1	1–2
2	29	1	3.3	1–25
3	65	12	15.5	1–36
4	113	8	6.6	1–5

Unsprayed

1	11	29	72.5	1–138
2	48	56	53.9	1–38
3	20	29	58.8	1–82
4	46	68	59.7	1–112

TABLE 2. AMOUNT OF INFECTION ON BRANCHES TAKEN FROM TREES IN ORCHARD A QUARTER OF A MILE FROM CEDAR BLUFF

Sprayed

Branch from tree number	Number uninfected leaves	Number infected leaves	Percent leaves infected	Number infections on various leaves
1	33	2	5.7	1–8
2	66	48	42.1	1–8

Unsprayed

1	43	54	55.7	1–33
2	39	48	55.2	1–28

TABLE 3. AMOUNT OF INFECTION ON BRANCHES TAKEN FROM TREES IN ORCHARD ONE MILE FROM CEDAR BLUFF[1]

Sprayed

Branch from tree number	Number uninfected leaves	Number infected leaves	Percent leaves infected
1	60	1	1.6
2	86	1	1.1
3	52	3	5.6
4	91	1	1.09
5	94	3	3.0
6	84	2	2.3
7	70	1	1.4
8	78	0	0.0
9	80	0	0.0
10	122	0	0.0
11	102	1	.09
12	30	0	0.0

Unsprayed

1	52	3	5.6
2	87	6	6.4
3	89	7	7.3
4	144	10	6.5

The above results are especially gratifying when one considers that the trees were sprayed only three times. As further evidence of the effectiveness of this method, and one which does not show in the tables above, it should be noted that on the sprayed trees there was a marked difference in the amount of infection in the tops of the older and higher trees and in the foliage on the lower half. On the lower half where the application of the spray was more thorough there were many branches practically free from the disease. This was not due to unlike exposures, for no such contrast could be detected on the control trees. The tables show very plainly the difference in the extent of infection at various distances from the cedars.

Too much emphasis cannot be laid on the fact that the spraying must be done at the critical time, which is within a few hours after the sporidia are formed on the cedar galls. For this reason the question might be raised as to the practicability of attempting to control the apple rust by spraying. However, most orchards are not so large but that the susceptible trees in it can be given the necessary attention at the proper time. Considering that this means not only a decided in-

[1] Note. Each infected leaf showed an average of from one to two infection spots.

crease in production and improvement in quality, but often the saving of the life of valuable trees, it would surely seem practical.

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CONTROL OF APPLE RUST BY SPRAYING

A PRELIMINARY REPORT

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(WITH PLATES XXIII AND XXIV)

The rust of apple has been one of the most serious diseases found in many orchards of West Virginia during the past few years. In the several places visited the varieties York Imperial and Rome Beauty have suffered greatest injury. The damage to fruit from the rust this year in one county where careful estimates were made, is placed at $75,000.

The red cedar also finds many portions of this state well suited to its requirements, and small areas occur frequently where the stand is practically pure, while scattering trees of this species are very numerous. Unfortunately, some of the best apple sections and typical cedar areas appear to coincide. This disease of cedar and apple has been known in the state for some years but it has been spreading rapidly into new localities during the past three seasons.

During the spring of 1912 this department began active field investigations as to the practicability of controlling apple rust by means of spraying. Personal observations on the disease, and results given by other investigators[1], led to the belief that it could be controlled by spraying at the proper time, hence a commercial orchard in the eastern part of the state was chosen as a desirable place in which to conduct some experiments and 38 trees were turned over to us for the work.

Of these trees, 19 were York Imperial and 19 Ben Davis. They formed a nearly square block in the orchard and were so located that one might reasonably expect them to receive uniform infection. The only spray materials applied to them during the season were those which we used in connection with this work.

The spray materials used were bordeaux mixture (3 lbs. copper sulphate, 5 lbs. lime, 50 gals. water), commercial lime-sulphur (1 gal. to 40 gals. of water) and atomic sulphur (7 lbs. to 50 gals. of water). Each tree was divided into four parts by imaginary vertical planes. One portion was left unsprayed and each of the other portions was treated with one of the above three spray materials. A large rubber blanket was spread over as much of the control portion as it would

[1] Emerson, R. A. Apple scab and cedar rust. Nebraska Agr. Exp. Sta. Bul., 88. 1905. Stewart, F. C. Notes on New York plant diseases, I. N. Y. Agr. Exp. Sta. Bul. 328 : 317–318. 1910.

cover while the tree was being sprayed. The spray material was applied under a good pressure, using a two cylinder hand pump. A large tag was placed on a branch near the center of each of the four portions after spraying and no tree was sprayed on more than one date.

Two trees, one Ben Davis and one York Imperial, were handled in this manner on April 22, 24, 29, May 4, 6, 8, 10, 13, 15, 18, 20, 22, 27 and 29. Other trees were similarly treated, but using only bordeaux and lime-sulphur, on April 18, 20 and May 1; while still others received only lime-sulphur and atomic sulphur on April 26 and May 2.

Before the last of May it became evident that the disease was very largely controlled on certain trees. The sprayed portions of the trees treated May 4 and 6 were especially free from rust and the portion sprayed with bordeaux was apparently the healthiest in each case. Early in June counts were made to determine the number of diseased and healthy leaves resulting from each treatment. The results secured from a portion of these counts are given below.

	York Imperial				Ben Davis			
Apr. 26	Control	Bord. mxt.	Lime-sul.	Atomic sul.	Control	Bord. mxt.	Lime-sul.	Atomic sul.
No. leaves counted	17	——	50	52	43	——	29	35
Per cent diseased	60	——	66	70	34.8	——	34.4	42.8
Apr. 29								
No. leaves counted	163	151	137	134	106	137	133	121
Per cent diseased	58.9	43.7	46.7	64.1	38.8	18.9	30.8	33
May 1								
No. leaves counted	125	140	139	——	135	139	141	——
Per cent diseased	80.6	65.7	51.1	——	50.3	20	27.6	——
May 2								
No. leaves counted	135	——	160	110	136	——	149	128
Per cent diseased	70.4	——	60.6	50	47.1	——	20.8	22.6
May 4								
No. leaves counted	145	156	147	147	130	185	121	158
Per cent diseased	86.9	23.7	53	35.4	37.7	8.1	16.5	19.6
May 6								
No. leaves counted	126	150	151	143	157	181	121	146
Per cent diseased	78.6	22.6	23.8	23.7	34.3	3.9	10.7	23.9
May 8								
No. leaves counted	138	160	145	154	175	127	142	138
Per cent diseased	63.7	63.1	75.8	42.9	39.4	29.9	37.3	32.6
May 10								
No. leaves counted	55	56	25	35	39	30	33	49
Per cent diseased	82	71.4	84	80	38.5	26.6	42.4	51.1

It will be seen from this table that all three spray materials applied May 6 were quite effective in controlling the rust. Trees sprayed

May 4 were fairly well protected, while those sprayed May 2 showed no benefit.

Notes as to the number of rust spots per leaf show that they were much less numerous on the sprayed portions of the trees treated May 4 and 6, but actual counts of them were not made.

The only marketable York Imperial apples in this orchard were secured from the tree sprayed May 6. Unfortunately, there was practically no fruit on either the bordeaux or control portions of this tree. It was also noted that the amount of fruit on many other York Imperial trees in that section of the state was quite uneven this season.

Counts of 600 apples taken from several unsprayed York Imperial trees showed an average of 85% of the fruit rusted, while there were practically no rusted fruits on the sprayed portions of the tree treated May 6.

These investigations have definitely shown that apple rust may be controlled by the spray mixtures in common use, *provided the application is made at the right time.* The time during which effective spraying may be done is, however, very short, and rainy weather is apt to occur during a portion of that time. There are three things which must be carefully considered when spraying for this disease. They are, condition of cedar apples, condition of apple foliage, and condition of weather. These three factors appear to be more closely related in apple rust infection than is the case with similar factors of many other diseases. Our investigations of the past season have shed some light on the correlation of these factors but more extended observations will be required before we would feel justified in reporting on this phase of the work or in making recommendations for the spraying of large orchards to control rust.

DEPARTMENT OF PLANT PATHOLOGY,
WEST VIRGINIA EXPERIMENT STATION.

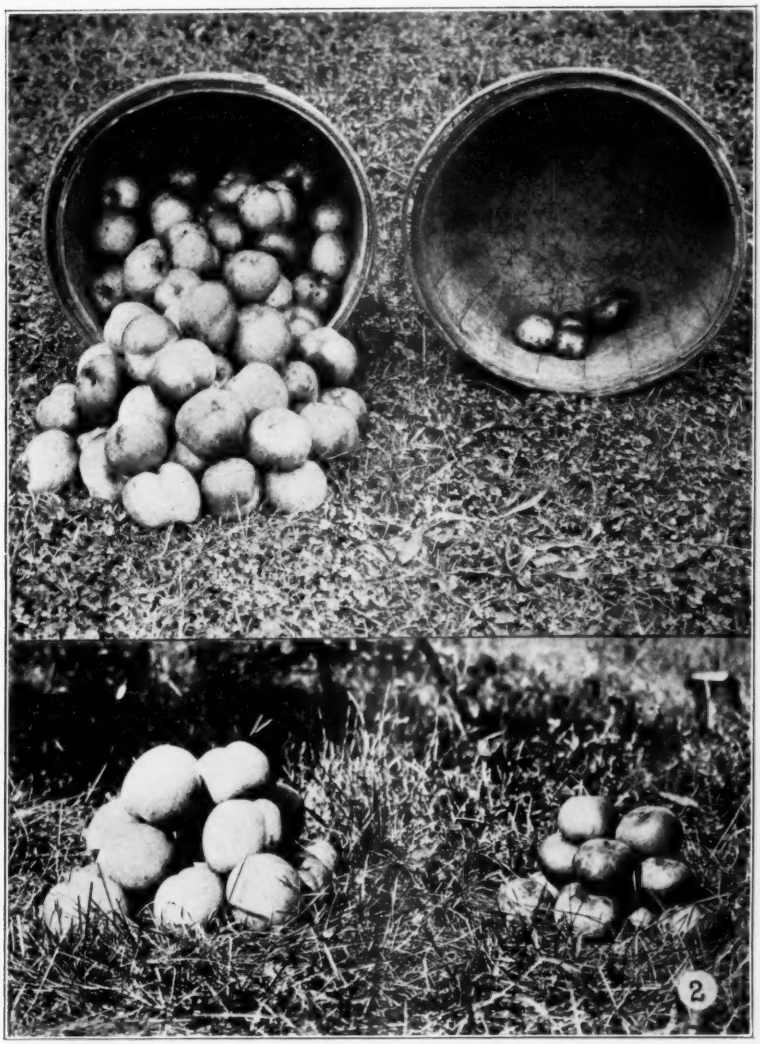

PLATE XXIII.—**Control of apple rust**

FIG. 1. Marketable fruit from sprayed portion of York Imperial tree sprayed May 6 at left. Total fruit from control portion of same tree at right.

FIG. 2. Eighteen typical apples from sprayed portion of York Imperial tree treated May 6, at left. Same number of typical fruits from an unsprayed York Imperial tree, at right.

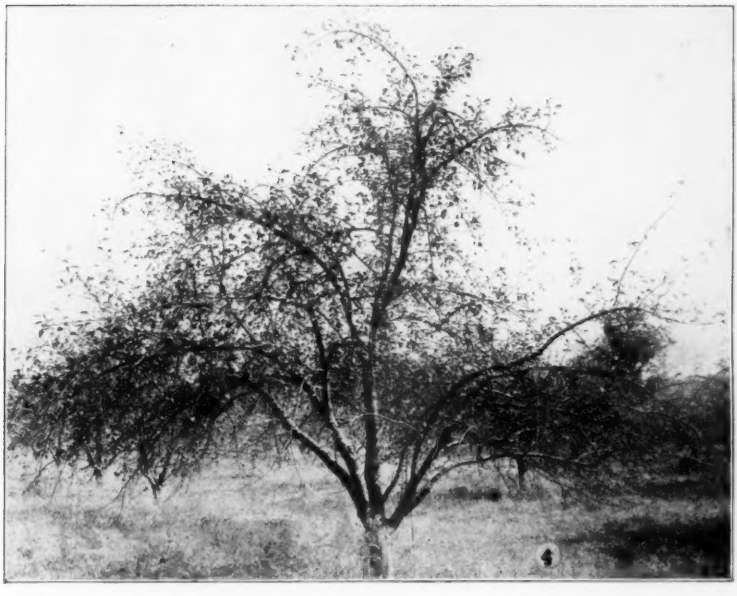

PLATE XXIV.—**Control of apple rust**

FIG. 3. York Imperial tree sprayed May 6.
FIG. 4. Typical York Imperial tree not sprayed for rust control.

ENDOTHIA VIRGINIANA

P. J. ANDERSON AND H. W. ANDERSON

In the last number of PHYTOPATHOLOGY there appeared an article by the writers[1] dealing with certain species of the genus Endothia. The "Connellsville fungus" was shown to be at best a weak parasite and to differ in a number of ways from the form known as *Diaporthe parasitica*. It also differs from the southern form which has been determined by Dr. Shear to be the real *Endothia radicalis*. The description of the Connellsville fungus which was omitted from our previous paper is given below.

Endothia virginiana n. sp.

Stromata gregarious, tubercular-pulvinate, round, elliptical or elongate (especially on older bark) erumpent, orange or orange-red, younger unexposed stroma often yellow, sub-pulverulent. Perithecia deeply buried in the stroma, with long black necks having distinct ostioles appearing as black dots over the flattened surface of the stroma or the necks exposed in the form of spine-like projections; perithecia 300–450 μ in diameter. Asci sub-clavate, 8-spored, aparaphysate, 32–37 μ in length. Ascospores irregularly monostichus or subdistichus, short-fusoid, hyaline, 2.4–3.1 x 6–7.2 μ, straight, 1-septate, very slightly constricted. Pycnidia as superficial chambers in the stroma or as single pear-shaped bodies borne on a scattered mycelium. Conidia hyaline, very small, bacilloid, 1.6 x 3.6 μ, oozing from the apex of the pycnidia in the form of yellow or orange thread-like masses.

Collected near Connellsville, Penna., by P. J. and H. W. Anderson, July, 1912. On dead bark and wood of *Castanea dentata* and *Quercus velutina*. Found commonly through southern Pennsylvania, West Virginia and Virginia.

The Latin description is as follows:

Stromatibus subrotundis v. irregularibus, confluentibus, erumpentibus, luteis v. aurantio-miniatis; peritheciis atris, longicolliis, 350 μ diam; collis nigris, demum prominulis; ascis clavatis, 32–37 μ longitudine, 8-sporis, aparaphysatis; sporidiis irregulariter distichis v. submonostichis, breve fusoideis, hyalinis, 1-septatis, vix constrictis, 2.4–3.1 x 6–7.2 μ Spermatiis minutissimis, oblongis, 1.6 x 3.6 μ, hyalinis.

Hab. in corticibus et ad ligna Quercus velutinae et Castaneae dentatae in America Boreali.

[1] The chestnut blight fungus and a related saprophyte. Phytopath. 2: 204–210. Oct. 1912.

Type specimens deposited in the Department of Plant Pathology of Cornell University (Number 6178). Duplicates in the private collection of the writers and in the collection of the Pennsylvania Chestnut Tree Blight Commission.

The measurements and the form of the asci and ascospores were based on the examination of freshly collected specimens. After the specimens have dried in the herbarium the asci contract and the ascospores often become distorted. This occurs in mature as well as immature specimens.

Endothia parasitica (Murr.) n. comb.

This combination is proposed as the name of the true chestnut blight organism. It was originally described by Dr. W. A. Murrill[1] as *Diaporthe parasitica* Murr. The writers are not the first to believe that the fungus belongs in the genus Endothia. Farlow[2] discusses at some length the reasons for placing it in that genus and also states that Saccardo and Von Höhnel concur in this opinion.

PATHOLOGICAL LABORATORIES OF THE PENNSYLVANIA
CHESTNUT TREE BLIGHT COMMISSION

[1] Torreya **6**: 189. 1906.
[2] Science, N. S., **35**: 717–722. 1912.

A NEW FUNGUS ON THE APPLE

JOHN W. ROBERTS

While investigating the cause of a canker disease of the Yellow Newtown Pippin a fungus was isolated which proved not only to be the cause of the disease but to be a species hitherto undescribed. It appears also on the foliage and perhaps occasionally on the fruit of the apple, in which cases it might easily be mistaken for a Phoma or Phyllosticta.

The globose rather large pycnidia of the fungus develop singly or in a raised stroma from one-half to one cm. across. A cross-section of a pycnidium shows a dark outer layer of large-celled fungous parenchyma ; inside this is a thick, lighter-colored layer of rather loose texture, making up the bulk of the pycnidial wall ; next is a densely-woven olivaceous or brownish layer ; and within is a closely woven hyaline layer. The interior of the pycnidium is usually more or less plurilocular through the upward growth or invagination of the base or by a protrusion interiorly from the side.

The spore masses when mature are extruded either in an indefinite cream-colored or yellowish mass, or in slender rope-like masses from one to four cm. long.

Spores are of two kinds : those called *A* spores by Diedicke[1] are spindle-shaped or nearly so, measure 7–10 x 3–4 μ, mostly 8 x 3 μ, contain two oil drops and are borne on cylindrical basidia measuring about 20 x 2.5 μ. They germinate readily in distilled water. Those which Diedicke designated as B spores and Shear[2] scolecospores, are thread-like, curved at the centre, hooked at one end, sigmoid or nearly straight, and somewhat attenuate. They vary considerably in size, the average being 28 x 1.5 μ. Repeated attempts have been made to germinate these spores but without success.

A pycnidium may produce spores all of one kind, but usually both kinds are found in the same pycnidium. A localization of the production of the two kinds of spores in separate portions of the same pycnidium, as mentioned by Diedicke, has never been observed.

The fungus seems to differ essentially from other fungi described as occurring on the apple. *Phoma mali*, shown by Lewis[3] to cause a

[1] Diedicke, H. Die Gattung Phomopsis. Ann. Myc. **9** : 8–35. 1911.

[2] Shear, C. L. The ascogenous form of the fungus causing dead-arm of the grape. Phytopathology **1** : 116. 1911.

[3] Lewis, C. E. Apple diseases caused by Coryneum foliicolum and Phoma mali. Maine Agr. Exp. Sta. Bul. 170. 1909.

canker and soft rot of the apple, resembles it in the size of spores and growth on certain media, but differs from it in the absence of secondary spores, in the structure of the pycnidium and in having less tendency toward stroma formation. The reactions of the two fungi toward certain culture media are also very different.

Accepting Saccardo's diagnosis of the genus Phomopsis and Diedicke's investigations of the genus, the fungus in question is clearly a Phomopsis. Of the described species of this genus the fungus resembles most closely *Phomopsis ambigua*. (Nits.) Trav. described as occurring on the pear and apple, but differs from it in having much larger secondary spores. It therefore seems best to the writer to describe it as a new species as follows :

Phomopsis mali sp. nov.

Pycnidiis subglobosis, sparsis, gregariis vel in stromate, nigris, carbonaceis, plurilocellatis, ostiolatis. , Sporulis subfusoideis, biguttulatis, continuis, hyalinis, 7–10 x 3–4μ. Basidiis subulatis, 20 x 2.5μ. Sporulis ordinis secundi filiformibus, uncinatis vel sigmoideis, attenuatis, 20–36 x 1.5μ.

Hab. in ramis truncisque et foliis Piri mali in Virginia, America boreali.

Pycnidia subglobose, scattered, aggregate or in a stroma, black, carbonaceous, plurilocular, ostiolate. Spores subfusoid, containing two oil drops, continuous, hyaline, measuring 7–10 x 3–4μ. Basidia awl-shaped, measuring 20 x 2.5μ. Secondary spores thread-like, hooked or S-shape, attenuate, measuring 20–36 x 1.5μ.

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CHESTNUT BLIGHT FUNGUS AND ITS ALLIES

G. P. CLINTON

It has recently been settled rather definitely by all who have studied the matter thoroughly that *Diaporthe parasitica* Murr. belongs more properly under the genus Endothia. Besides the true chestnut blight, there are in this country at least two other forms of Endothia, the linear-spored form, first pointed out by Farlow, and the narrowly-oval-spored form, first identified by the writer; while the broadly-oval-spored form is that of the true chestnut blight of Murrill. The Andersons, in their recent paper in PHYTOPATHOLOGY, also bring out these differences in the ascospores. In a paper recently or soon to be published, the writer has called these forms (1) *Endothia radicalis* (Schw.) Farl.; (2) *Endothia gyrosa* (Schw.) Fr. (*E. virginiana* Anders.); and (3) *Endothia gyrosa* var. *parasitica* (Murr.) Clint. So far as known, only the second of these has been found in Europe, where it occurs on similar hosts, Castanea and Quercus. The writer wishes to present in this paper information concerning certain specimens recently brought to light by him in the Schweinitzian collections at the Philadelphia Academy of Science, also to give the present known distribution and hosts of these three forms.

Farlow, Shear and the Andersons have examined the specimens of *Sphaeria radicalis* and *S. gyrosa* in the Philadelphia Academy of Science, and they all found that the single specimen of the former, while a true Endothia, had only the Cytospora stage. The one specimen found under the name *S. gyrosa*, they agree, is not an Endothia, but a species of Nectria. The writer's examination confirmed these conclusions. He is indebted to Mr. Stewardson Brown, the curator, for the privilege of examining the regular set and also all the miscellaneous unmounted specimens of Schweinitz. The Schweinitzian type specimens as they now stand were remounted by a special committee on fungi, some time after their presentation to the Academy upon Schweinitz's death. This committee mounted each specimen exposed on a piece of paper on which they wrote the name, locality, and number referring to description in Schweinitz's N. A. F. These were then placed in order on the inner halves of folded sheets having ten spaces marked off in two rows. Where there was a specimen missing, the corresponding space was left blank. These sheets were placed in portfolios numbered according to Schweinitz's classification.

Schweinitz originally had his specimens in folded packets which had on the outside a list of the species, arranged according to his N. A. F. Several of these packets, each containing from twenty-five to fifty small loose envelopes, were tied together in a portfolio. On the outside of the small envelopes containing the specimens, in Schweinitz's own handwriting, were the name of the fungus and other meager data, usually locality and host. Fortunately, the committee, on remounting these specimens, did not destroy the original envelopes; they left them just as Schweinitz had them, and sometimes even left part of the specimens in them.

Referring back to these envelopes, we found that the one originally containing the specimen of *Sphaeria radicalis* had on it in Schweinitz's writing the name of the host,—"in rad. Quercuum," which had not been copied on the label with the type. This clears up a misunderstanding, since when Schweinitz published his description of this species he gave the host as roots of Fagus. The host is evidently Quercus and not Fagus, for no one since has found the fungus on Fagus, while Quercus is a common host.

Looking up the original envelope of *S. gyrosa*, we found this threw some light upon the New England specimen that Schweinitz received from Torrey, mentioned under No. 1431 of his N. A. F. as *S. gyrosa*. This envelope contains in Schweinitz's writing in faded ink the following: "*Sphaeria gyrosa* L. v. S. Salem." In darker ink, evidently written some years after, the words were added:—"& Nov. Anglia Tr. cum Tuberc. velutipes in Nov. Anglia." This shows that Schweinitz when he received the specimen from Torrey put it in the original envelope of the Salem type, which he had then evidently lost, and so mistook the Torrey specimen to be the same, and added the above note, which applies to the specimen now mounted, which the committee labeled,—"1431-283. Syn. Fung. *Sphaeria gyrosa* Schw. Salem-N. Engl."

The question arises, what became of Schweinitz's original type specimen of *S. gyrosa* from Salem. The writer thinks he has solved this mystery. Specimens are very easily lost out of such envelopes as Schweinitz used. He, in common with botanists of his time, made exchanges with numerous other collectors, hence the specimens in the Fries herbarium at Upsala and the Curtis herbarium, now at Harvard. Apparently one of the best sets that Schweinitz gave to another botanist was that presented to Collins of Pennsylvania, and this included a nearly complete set of his Sphaerias, as well as some other genera. The small envelopes which contained these specimens were enclosed in neat white packets, better than Schweinitz's own, on the outside of which

appeared in Schweinitz's own writing the list of contained species and also those of his N. A. F. that were missing. Both *S. gyrosa* and *S. radicalis* are listed with the missing species. However, in the envelope *Sphaeria enteromela*, No. 12 of Packet 1, I found, besides a typical specimen of *S. enteromela*, that agrees with the three specimens mounted in Schweinitz's collection and exactly matches the wood of one of them, a fine specimen of Endothia (apparently on oak, but unfortunately only in its Cytospora stage) which it seems to me may be the missing specimen of *S. gyrosa* lost from Schweinitz's own collection. Mr. Brown informed me that this collection came into the possession of the Academy after the death of Collins in 1831; apparently it had not been seen by those who had examined the Schweinitz specimens, since Mr. Brown had overlooked this collection previously.

Besides this set, there are also numerous other specimens of Schweinitz that have never been mounted, and some of these may perhaps be species missing in Schweinitz's own herbarium, though we were unable to find among them any additional specimens of *S. radicalis* or *S. gyrosa*. However, in Schweinitz's own set we did find under a different name another specimen which is merely the Cytospora (Endothiella) stage on wood of *Endothia radicalis*. This is labeled,—"840-97. Syn. Fung. *Peziza cinnabarina* Schw. Salem." In his description in N. A. F., Schweinitz states that this is on the wood of Liquidambar, a host on which Ravenel, Earle, and Ellis have since collected *Endothia radicalis*. Schweinitz originally (Syn. Fung. Car. No. 1193) apparently thought this the same as *P. flammea*, Consp. Fung. N. p. 319 t. 1. f. 6. Saccardo later (Syll. Fung. 8: p. 399) placed it among imperfectly known species of Lachnella, *L. cinnabarina*.

Through the kindness of Dr. Murrill, the writer has also examined the specimens of Endothia in the New York Botanical Garden. From these sources, the writings of others, correspondence with Professor Farlow, and our own observations, we have been able to gain a fair idea of the hosts and distribution of the three forms as they occur in this country. In addition to these three, a fourth form has been described by Earle (Muhl. 1901: 14) from Porto Rico, on bark of fallen trees. Specimens of this species show very long, exposed, beak-like ostioles, with the stroma reduced to the minimum; while the most northern of our Endothias shows a maximum development of the stroma, with the least exposure of the ostioles. No doubt with the latter these are adaptive characters giving greater protection to the ascospores against a colder climate, since specimens collected in the Southern States sometimes show a gradation in these characters between the northern form and that found in Porto Rico.

(1) The linear-spored Endothia, *E. radicalis* (Schw.) Farl.

Perhaps most of the specimens called *Endothia gyrosa* in American herbaria come under this species, though it is impossible to say definitely, since many of them are represented only by the Cytospora stage. So far as we have seen ascospore specimens, these have come from the South, and give a known distribution from Mississippi and Florida to North Carolina. The following are referred definitely to it: On Quercus sps., La. (Farlow), Miss. (Earle, Tracy), Ala. (Earle), Fla. (Calkins, Thaxter, Mrs. Curtis), So. Car. (Ravenel, Rolfs, Hall, Jenkins), No. Car. (Schweinitz type *S. radicalis*, Anderson); on Liquidambar sp., Ala. (Earle), So. Car. (Ravenel), No. Car. Schweinitz) type *P. cinnabarina*), N. J. (Ellis). There are also Cytospora specimens of either this or the next species in the New York Botanical Garden herbarium on Quercus sps. from Ohio (Morgan), Kan. (Swingle), and Calif. (*Q. agrifolia*, Baker); and on Vitis sp. from Ala. (Earle and Uuderwood). The writer has had under observation for nearly a year a fungus on the roots of *Quercus rubra* in Connecticut that has failed to produce the asco-stage on its host, but when grown in cultures looks like this species. Apparently this species has not yet been found in Europe.

(2) The narrowly-oval-spored Endothia, *E. gyrosa* (Schw.) Fr.

So far as we have learned, this fungus occurs from North Carolina to southern Pennsylvania The hosts and distribution are as follows: On *Castanea dentata*, No. Car. (Clinton), Tenn. (Clinton, Anderson), Va. (Clinton, Rankin, Spaulding, Anderson), W. Va. (Giddings, Anderson), Penn. (Detwiler, Anderson); on *Quercus alba*, D. C. (Clinton), No. Car. (Clinton); on *Q. velutina*, D. C. (Clinton); on Quercus sps., D. C. (Clinton), Va. (Clinton), No. Car. (Clinton); on Fagus [?] and Juglans [?], No. Car. (Schweinitz type *S. gyrosa*). It also occurs in France, Italy, Switzerland, and perhaps other European countries, on species of chestnut and oak. Specimens from Germany and further north seem to produce the Cytospora stage only. Saccardo gives the hosts and distribution as follows:—*Endothia gyrosa* on Æsculus, Alnus, Carpinus, Castanea, Corylus, Fagus, Juglans, Quercus, and Ulmus, from North America, Europe, Ceylon and New Zealand," but just how many of these really refer to the species under consideration is a question that only an examination of asco-material can determine.

(3) The broadly-oval-spored Endothia, *E. gyrosa* var. *parasitica* (Murr.) Clint.

This is the true chestnut blight of the northeastern United States. It seems to be the most northern of the three, and as yet has not been recognized outside of the United States. Its hosts and distribution here

as determined by the writer and others are as follows:—On *Castanea dentata*, N. H., Mass., R. I., Conn., N. Y., Penn., N. J., Del., Md., D. C., Va., W. Va.; on cultivated *C. sativa*, not uncommon, and on *C. crenata*, rarely, in Conn., N. Y., and probably elsewhere; also reported by Dr. Morris as rarely on cultivated *C. pumila* and *C. alnifolia* in Conn.; on *Quercus alba*, Conn. (Clinton); *Q. rubra*, Conn. (Clinton): *Q. velutina*, Penn. (Fulton, Detwiler).

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ETIOLOGY OF CROWN GALLS ON SUGAR BEET

ERWIN F. SMITH

In the *Zeitschrift für Zuckerindustrie in Böhmen* for October and November, 1911, is a paper by Dr. Karl Spisar, entitled *Ueber die Bildung des Zuckerrüben-Kröpfes*, in which he maintains the beet gall to be due to mechanical injuries, but hedges to the extent of saying: "Damit will ich jedoch nicht gesagt haben, das die Kröpfe einzig und allein nur auf diese Art und Weise entstehen müssten." The paper is the result of laborious experiments covering a period of years. The first nine pages deal with the general history of crown galls. The remaining thirty-two pages are devoted to the author's own work.

Spisar had over 5,000 beets in his experiments, on which individual protocols were kept, but if he had had 50,000 with similar results it would not, in my opinion, establish his contention, for several reasons.

Of our own work he says that we failed to obtain any experimental proof of the bacterial origin of the gall on the sugar beet (ignoring the meager but positive proofs shown in Plate 36 of Bur. Pl. Ind. Bull. 213, and the corresponding statements in the text). He says further that our tumors on sugar beets have a different appearance from his and are probably of entirely different origin. The only differences he points out are the accidental and trivial ones that those we figured showed more than one center of growth, and are attached by a broader base. He states (p. 8) that his galls are *always* attached to the beet by a neck, but soon after contradicts this statement both in text and figures (pp. 15, 16, and 17). He also contradicts it on page 69 where he says: "The form, size and color of the galls, as well as their location on the root, may be extremely varied. * * * No purpose is served by grouping the beet galls according to their forms, as various authors have done, for we know now, that all hitherto observed beet root galls have the same origin, that they are *callus formations* which have arisen through *wounding* of the beet root, in which the heaping up of food plays a part; the form and size of the gall is not decisive." His figures resemble ours.

In reading Spisar's paper one is struck first of all by the small number of well-developed galls he obtained, *i. e.*, from the 5,000 wounded plants "5 large galls and 12 beets with almost full-grown galls." This is not a very remarkable showing, considering the great number of wounds made. It is true that on one of his fields (sandy land) he reckons 21 per cent. of successes, on another (moderately fertilized) 53 per cent., and on a third (heavily manured) 76 per cent.,

but this is only by counting as galls all slight over-growths, to which he admits the beet is very subject (p. 67). Indeed, Spisar speaks of his large per cent as beets, *bei denen jedoch die Dimensionen der Kröpfe die Grösse einer gewöhnlichen Kallus nicht ueberschritten hat.* Apparently if the callus was not corked over when examined it was reckoned as a gall.

Because he came to the conclusion that one wound was not sufficient, he made two parallel ones, cross or longitudinal, of varying depths and breadths, for the most part in the crown. The galls sometimes grew out of the whole cut surface, sometimes out of a single limited portion of it. The former were especially common. The author states that by similar wounding he has produced "Wurzelkröpfe" on the carrot.

The most remarkable fact, however, in his paper, and the most unaccountable one, admitting the author's premises, as well as the one to which he pays least attention, is his failure to obtain galls on any of the wounded beets in a fourth field. Concerning the experiment in that field, at Úholičky near Prag, he says: "I must at the same time remark that it yielded no satisfactory results, for beyond the ordinary healing of the wounds on the beet roots operated upon, true Kröpfe were not observed on them."

What Spisar fails to explain is why, out of hundreds of thousands of wounds occurring naturally in fields of sugar beets between spring and autumn, as the result of cultivation, insect injuries, unequal growth, etc., only now and then one results in a gall. The best he can do is to offer the following: "Auf Grund solcher Erfahrungen müssen wir annehmen, dass in solchen Fällen noch einige zur Kröpfbildung unbedingt notwendige Bedingungen fehlten."

In another place he says: "I am of the opinion that every sort of wounding can lead to gall-formation, if in addition the *disposition* is present."

I quote also as follows: "The facts cited show sufficiently it is hoped that the cause of the gall-formation in the sugar beet is to be sought in the wounding of the tap root." But further on he says, as earlier in the paper: "Nevertheless I will not presume that the Kröpf of the sugar beet can arise *only* in the manner indicated, viz., through *wounding*." And further: "The gall-formation depends first of all upon the nature of the soil in which the beet-plants grow, then *on the amount of moisture in the same, on the age of the root*, and on the *size of the wound*. But the internal conditions must not be overlooked for it happens very often, that in spite of all favorable external factors no gall arises. Perhaps there are involved other yet unknown factors,

which favor or restrict gall-formation." And again: "Dass die innere Disposition für die Kropfbildung wichtig ist, bleibt unbestreitbar; näheres lässt sich darüber jedoch nicht sagen."

The following statements are also interesting: "It is generally known that in one year the beet root gall occurs abundantly, again in other years it is rare! Cases are known where on one field galled beets have been numerous, while on another in the vicinity none have appeared. H. Briem has also called my attention to this fact; in the year 1909 there were no galled beets in the beet fields in Úholičky, while close by, at Jenč, they were observed in numbers."

If we admit, for argument's sake (and there is no evidence to the contrary) that a gall-causing organism, liable to be introduced through wounds, was present in the three fields first mentioned, then Spisar's argument falls to the ground. Moreover, why did no galls appear on the wounded beets in the field at Úholčiky? Answer: Because the causal organism was not present in the soil of that field.

To such objections I cannot see that his paper affords any satisfactory answer. We may admit, for argument's sake, and this I am quite willing to do, that Spisar's galls on beets are not of bacterial origin, and even that they are due simply to wounds, leaving the question an open one, but the evidence in favor of these two contentions (whether feeble or strong) is not materially strengthened by Spisar's experiments.

All that Spisar has really established beyond cavil is that the crown gall sometimes follows wounds, a fact already known.

PHYTOPATHOLOGICAL NOTES

Aerial Isolation and Inoculation with Pythium debaryanum. In October, 1911, *Pythium debaryanum* was isolated by exposing plates at a height of thirty feet above ground. Inoculations with cultures of the fungus thus obtained from the air were made by puncturing with a needle individual seedlings grown on heat-sterilized soil, and applying small masses of mycelium directly to the seedlings. Results were as follows:

Picea canadensis: 100 inoculations, 15 effective.

Pinus ponderosa: 100 inoculations, 22 effective.

Inoculations were also made with cultures secured from other sources, by applying masses of mycelium to marked points in trays of sterilized soil on which coniferous seedlings had been sown. Results were as follows:

Culture taken from cabbage plants:

Picea canadensis: Inoculation at 50 points; 24 effective.

Pinus ponderosa: Inoculation at 50 points; 14 effective.

Cultures taken from *Salsola tragus:*

Picea canadensis: Inoculation at 50 points; 32 effective.

Pinus pondorosa: Inoculation at 50 points; 20 effective.

Cultures taken from radish:

Picea canadensis: Inoculation at 50 points; 18 effective.

Pinus ponderosa: Inoculation at 50 points; 7 effective.

No infection occurred except at points of inoculation.

JULIUS V. HOFMANN

Dr. E. S. Reynolds, recent assistant professor of botany in the University of Tennessee, has been appointed associate professor of botany in the University of North Dakota. Dr. Ira H. Cardiff, recently professor of biology in Washburn College, has been appointed associate professor of plant physiology and bacteriology in the State College of Washington. Dr. B. M. Duggar, recently professor of plant physiology in Cornell University, has been appointed professor of plant physiology and applied botany in Washington University, succeeding Dr. Geo. T. Moore, who is now Director of the Missouri Botanic Garden. Dr. H. B. Humphrey, plant pathologist of the Washington State Experiment Station, has been appointed head of the department of botany of the college and station. Mr. C. P. Smith, recently instructor in plant pathology in the College of Agriculture of Cornell University, has been appointed associate professor of botany in the Maryland Agricultural College and seed analyst to the Maryland State Board of Agriculture.

John S. Boyce, and Miss Ruth M. Bates, recently graduate students in the University of Nebraska, have been appointed scientific assistants in Forest Pathology in the Bureau of Plant Industry. Mr. Boyce's address is care of Forest Service, San Francisco, California; Miss Bates' address, Forest Products Laboratory, Madison, Wisconsin. Dr. I. E. Melhus, recently instructor in plant pathology in the University of Wisconsin, has been appointed pathologist in the Bureau of Plant Industry to take charge of potato disease investigations. Mr. M. Shapovalov, recently a graduate student in the University of Wisconsin, has been appointed assistant in plant pathology in the Maine Experiment Station.

Mr. A. B. Brooks, former State Forester of West Virginia, and Mr. C. L. Brooks, have been appointed by the Office of Forest Pathology of the U. S. Department of Agriculture in cooperation with the Department of Plant Pathology of the West Virginia Experiment Station to conduct investigations concerning the Chestnut Bark Disease in this State. They have already found a badly diseased area at the extreme eastern border of the State.

Prof. T. J. Burrill has retired under a Carnegie pension after active service in the University of Illinois for over forty years. At its last commencement the University of Illinois conferred on him its honorary degree of Doctor of Laws. Dr. Burrill is widely known for his pioneer work on pear blight, having been the first scientist to recognize a bacterial disease in plants.

NOTICE TO MEMBERS. The fourth annual meeting of the American Phytopathological Society will be held in conjunction with the American Association for the Advancement of Science, at Cleveland, Ohio, December 31, 1912, to January 3, 1913. The members of our Society are invited to meet with Section G., A. A. A. S., Tuesday, 2:30 P. M. Dec. 31. Our sessions will be held in the City Hall Wednesday, Thursday and Friday. The pathological exhibition, which was such an interesting and successful feature of the Washington meeting will be continued at Cleveland. Correspondence and exhibits should be addressed to Mr. John Boddy, City Hall, Cleveland, Ohio. Facilities will be provided for displaying exhibits of all kinds.

C. L. SHEAR, Secretary-Treasurer

REVIEW

Smith, Erwin F., Brown, Nellie A., and McCulloch, Lucia. The structure and development of crown gall: A plant cancer. U. S. Dept. Agric., Bur. Pl. Ind. Bul. 255. 109 plates, 2 text-figures. June 29, 1912.

This bulletin treats of the histology of crown gall. It is noteworthy in that it is illustrated by a remarkable series of plates all reproduced from photographs and most of them from photomicrographs, aiming to present the original evidence upon which the conclusions are based. It is assumed that the reader is conversant with the earlier bulletin from the same laboratory (No. 213) concerning the general nature of this disease and the bacterial parasite which causes it. The parasite has been isolated from 24 species of plant and proved to be either one polymorphic species (*Bacterium tumefaciens*) or several closely related ones. The infectious nature of the organism has been shown by hundreds of inoculations.

The morphology and biology of the tumor receive special consideration. The matters of major interest are, (1) The development of secondary tumors, which in some cases arise at considerable distance from the primary and may be in other organs; e. g., the primary tumors on the stem and the secondary on the leaf. (2) The occurrence of tumor strands extending from the primary tumor in various directions through the normal tissue. In the substance of these deep-lying strands the secondary tumors develop and rupture their way to the surface. The secondary tumor shows the structure of the primary tumor, even when located on another organ; e. g. if the primary is on the stem and the secondary on the leaf, this latter shows a stem type of structure. (3) The parasite has been shown to occur within the cells, not only in the primary tumor but also in the secondary tumor and in the connecting strand. The parasite does not occur in all of the cells in the diseased tissue and its differentiation has proved extremely difficult. After trying in vain the various aniline dyes, the authors accomplished this by gold chloride impregnation.

Following these established facts, the authors suggest two hypotheses of interest. The first aims to account for the variable character of the galls. It is suggested that this is determined by the character of the tissue first infected. If this consists of meristem giving rise to tracheids, a hard gall is developed, whereas, if the cells first infected are mother cells of the medullary rays a soft gall may arise. In the second place it is suggested that the stimulus to hyperplasia may take place through the delicate adjustment of opposing forces, first the parasite and then the host having the advantage. The details of the

hypothesis by which this is explained are developed in an ingenious manner with evidence from cultural studies in their support. These include the development by the bacteria of an acid which kills some of the organisms. From the dead bodies endotoxins escape and these or other products stimulate nuclear divisions of the host cells. These nuclear changes in turn may incite the dormant bacteria into renewed activities in the daughter cells until once more inhibited or killed, whereupon the daughter cells again divide. Thus it is evident that, while it is the rapidly proliferating host cells which do the mischief, they are impelled to this pathological behavior and continued division by the stimulus of the invading organism, which in turn passes into the daughter cells to repeat the process indefinitely.

The authors point out that in these neoplastic growths crown gall stands in the same category as animal tumors. They term it, therefore, a plant cancer, although not implying that the parasite of crown gall has anything to do with human cancer. Whether or not investigators of human cancer accept the authors' belief that human cancer is due to a cell parasite, they must give careful consideration to this evidence and to the methods of research upon which this is based. We can at least be sure that this publication must reopen the whole question of parasitism in relation to cancer, and secure its critical reinvestigation along the line suggested by Dr. Smith's methods. Such an outcome will justify the statement of Dr. Galloway, in his letter of transmittal, that the work is of "the first magnitude in pathology."

L. R. JONES

[PHYTOPATHOLOGY, for October, 1912 (**2**: 181–216, *pls.* XVII–XX) was issued October 30, 1912.]

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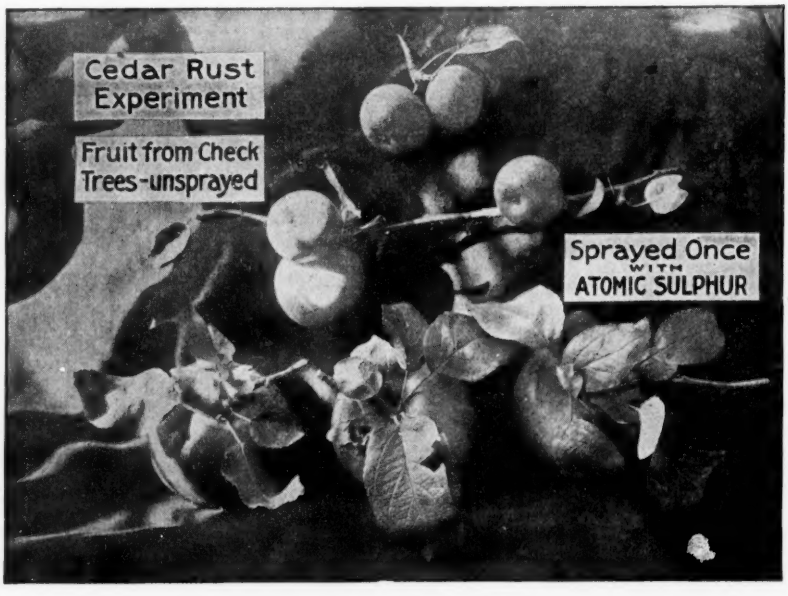

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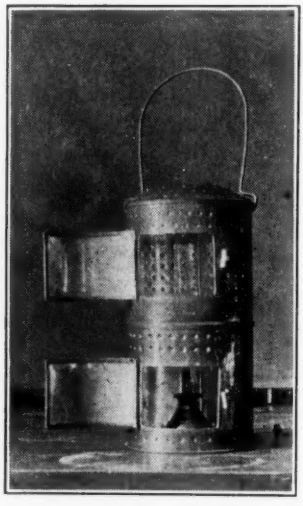

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